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DYNAMIK DER STICKSTOFFMINERALISATION IM ORGANISCHEN WALDBODENMATERIAL

II. EINFLUSS DES STICKSTOFFGEHALTES AUF DIE MINERALSTICKSTOFF-NACHLIEFERUNG

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Die Frage nach der Abhängigkeit der Stickstoffmineralisation vom Stickstoffgehalt des untersuchten Materials ist bereits häufig behandelt. Aus der umfassenden Darstellung von Harmsen u. Van Schreven [8] ** geht hervor, dass viele Arbeiten einen Zusammenhang zwischen C : N-Verhältnis (bzw. Stickstoffgehalt der organischen Substanz) und Mineralstickstoff-Nachlieferung erkennen lassen: Bei engem C : N-Verhältnis ist die Nachlieferung gross, bei weitem C : N-Verhältnis verringert sie sich stark. Diese Gesetzmässigkeit gilt in erster Linie für die Mineralisation von zum Boden zugegebenem organischen Material wie z.B. landwirtschaftlichen Ernterückständen oder Waldstreu (Wittich [29 30]). Sie spielt daher bei der Kompostierung eine Rolle: Nur solange das C : N-Verhältnis im Kompost kleiner ist als 20 bis 25 (N-Gehalt über 1,5 bis 2% der org. Substanz), ist mit einer N_{min}-Anlieferung zu rechnen; sonst wird infolge hoher Immobilisation gar kein Mineralstickstoff angeliefert. Aber auch im organischen *Boden*material besteht vielfach eine Abhängigkeit der N_{min}-Anlieferung vom C : N-Quotienten. Sie tritt hier allerdings nicht regelmässig auf [8], was wahrscheinlich mit der verschieden guten mikrobiellen Angreifbarkeit des untersuchten organischen Materials zusammenhängt [8 22].

Da mit Waldbodenmaterial erst relativ wenige Untersuchungen

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** Da diese ausführliche Übersicht die wichtigste Literatur annähernd vollständig berücksichtigt, kann hier an Stelle von Originalzitaten auf sie verwiesen werden.

vorliegen, und teilweise uneinheitliche Ergebnisse erzielt wurden, stellten wir uns die Frage, in welchem Umfang bei organischem Bodenmaterial von verschiedenen Waldstandorten Bayerns ein Zusammenhang zwischen dem Stickstoffgehalt und der Mineralstickstoff-Nachlieferung besteht. Dabei prüften wir zunächst die Abhängigkeit der Mineralstickstoff-Nachlieferung vom C : N-Verhältnis des Materials (Abschn. 1), beobachteten den Einfluss von Mineralstickstoff-Zusatz auf das Mineralisationsgeschehen (Abschn. 2) und untersuchten schliesslich, inwieweit die mikrobielle Angreifbarkeit des organischen Materials eine Abhängigkeit der N-Mineralisation vom Stickstoffgehalt überlagert (Abschn. 3).

VERSUCHSMATERIAL UND METHODIK

Das Versuchsmaterial stammt von verschiedenartigen Waldstandorten Bayerns. Sie liegen zum grössten Teil im Bereich der staatlichen Forstämter (vergl. Abb. 1 bei Zöttl[36]). Näheres wurde bereits in der vorhergehenden Arbeit [37] geschildert oder ist aus den Unterschriften der Figuren zu entnehmen.

Wir führten die folgenden Untersuchungen in Brutversuchen durch. Das hierbei angewandte Verfahren schilderten wir bereits eingehend [35 37]. Auch bezüglich der chemischen Analysenmethoden sei auf die vorhergehende Arbeit [37] verwiesen.

Zeitkurve der Kohlendioxyd-Produktion

Die Messung der CO_2-Produktion nahmen wir ebenfalls nach der bereits geschilderten Methode vor.[37] Da sich der Verlauf der CO_2-Kurven bei Proben ohne Zusätze grundsätzlich gleicht, sei nachfolgend zunächst der typische Verlauf der CO_2-Produktion während des Brutversuches geschildert und interpretiert. Wir vermeiden so spätere Wiederholungen.

In den ersten Tagen nach Versuchsbeginn steigt die CO_2-Produktion laufend an, bis sie nach etwa ½–2 Wochen einen Gipfel erreicht *. Sodann fallen die Kurven verschieden langsam wieder ab. Das Atmungsmaximum spiegelt wohl die unter den günstigen Brutbedingungen rasch einsetzende Verstärkung der mikrobiellen Aktivität und Vermehrung wider. Auf Grund der Ergebnisse von Woolridge [32], Stevenson [25] und Birch [2] dürfen wir annehmen, dass vor allem eine gesteigerte Umsatzaktivität der neu entwickelten Mikroorganismen dieses CO_2-Maximum hervorruft. Den grössten Anteil am ersten Aufleben der Organismentätigkeit dürften die Bakterien haben. Dies geht aus parallel laufenden Bestimmungen von CO_2-Produktion

* Der Kurvenanstieg zu diesem Gipfel ist natürlich nur erkennbar, wenn man die ersten CO_2-Messungen bereits unmittelbar nach Versuchsbeginn vornimmt. Bei unseren Versuchen fällt die erste CO_2-Messung jedoch vielfach schon in die Zeit, in der das Maximum bereits erreicht ist.

und Mikroorganismenzahl in Brutversuchen von Neller [20], Vandecaveye[27], Jensen [11] und den Überlegungen von Zöttl [35] hervor.

Infolge des steigenden Verbrauches von mikrobiell leicht verfügbarem C-Material nehmen die Atmungswerte nach Erreichen eines milieubedingten Maximums wieder ab. Das Absinken der CO_2-Produktion kann allmählich und asympthotisch bis auf sehr geringe Werte erfolgen [18 28 21]. Wie die Figuren der folgenden Abschnitte zeigen, wird dieser Kurvenabfall jedoch manchmal von einem zweiten Gipfel unterbrochen. Ein solcher Wiederanstieg ist auch bei den mit unserer Methodik durchgeführten Brutversuchen von Ehrhardt [5] zu finden.

Vandecaveye [27] und Bingeman *et al.* [1] konnten nachweisen, dass in länger dauernden Brutversuchen bedeutende Fluktuationen der einzelnen Mikroorganismengruppen stattfinden. Auf das rasche Aufleben der Bakterientätigkeit folgt häufig ein zweites Maximum, das Aktinomyzeten und höhere Pilze hervorrufen. Viele unserer CO_2-Zeitkurven gleichen im Verlauf den Atmungskurven bei Vandecaveye [27], der zugleich die Keimzahlen der Mikroorganismen erfasste. Auch in unserem Waldhumusmaterial tritt vermutlich eine ähnliche Verschiebung in der Aktivität der einzelnen Mikroorganismengruppen ein. Diese Deutung des zweiten CO_2-Gipfels durch die im Brutversuch später relativ stärker einsetzende Pilztätigkeit erfährt durch die im Abschnitte 21 (S. 194) geschilderten Ergebnisse eine gute Stütze. Ausserdem spricht für eine solche Auslegung, dass bei Mullhumusproben mit überwiegender *bakterieller* Tätigkeit die CO_2-Werte keinen zweiten Gipfel erreichen.

ERGEBNISSE UND DISKUSSION

1 *Abhängigkeit der Mineralstickstoff-Anhäufung vom Kohlenstoff: Stickstoff-Verhältnis des Bodenmaterials.*

11 Einfluss der Brutdauer auf die Beziehung zwischen Kohlenstoff:Stickstoff-Verhältnis und Mineralstickstoff-Anhäufung.

Die Zeitkurven der N_{min}-Anhäufung * weichen in den ersten Versuchswochen vielfach mehr oder minder stark von einem linearen Anstieg ab [35]. Deshalb sind je nach der gewählten Bebrütungsdauer verschiedene Beziehungen zwischen dem C : N-Verhältnis und der angelieferten N_{min}-Menge zu erwarten. Da nach Überwindung der Anlaufperiode ([37]; Fig. 4 in [35]) fast alle unsere N_{min}-Anhäufungskurven etwa geradlinig ansteigen, schien es zweckmässig, die nach relativ kurzer Versuchsdauer angehäuften N_{min}-Mengen zur Charakterisierung heranzuziehen. Wir erfassen

* N_{min} = Mineralstickstoff (NH_4-N + NO_3-N).

dann gerade bei ungünstigem Rohhumus die geringen Werte der Anlaufzeit, in der die Mikroflora sich noch nicht an die gegenüber dem Standort wesentlich günstigeren Brutbedingungen angepasst hat. Dies ist nicht mehr der Fall, wenn man die N_{min}-Werte nach vielen Wochen Bebrütungsdauer heranzieht. Etwaige Beziehungen können sich dann verwischen.

Wir prüften die Frage nach der geeignetsten Bebrütungsdauer an 40 Rohhumus- und Moderproben, die teilweise eine ausgeprägte Anlaufperiode der N_{min}-Anhäufung erkennen liessen. Die folgenden Figuren 1–3 zeigen die Korrelation zwischen dem N_t-Gehalt * der organischen Substanz und der N_{min}-Anhäufung nach 3, 6 und 15 Wochen Brutdauer. Alle N_{min}-Werte sind in % des Gesamt-Stickstoffs der Probe ausgedrückt, da nur so ein Vergleich von Proben mit verschiedenem N_t-Gehalt möglich ist.

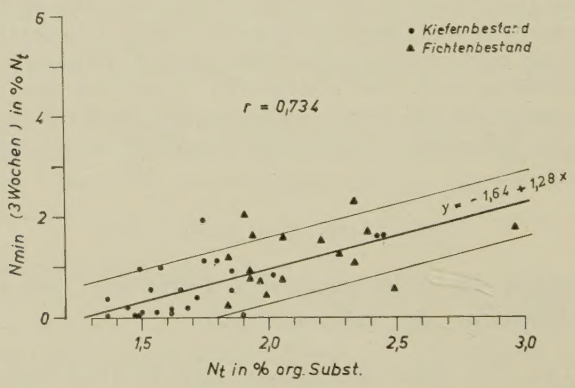

Fig. 1. Beziehung zwischen N_t-Gehalt der organ. Substanz und N_{min}-Anhäufung nach *3 Wochen Bebrütung* bei Rohhumus- und Moderproben.

Trägt man die nach *3 Wochen Bebrütung* festgestellten N_{min}-Werte auf (Fig. 1), so erhält man einen korrelativen Zusammenhang zwischen N_t-Gehalt der organischen Substanz und N_{min}-Nachlieferung ($r = 0{,}734$; mit 99,99% gesichert). Mehrere Proben weisen nach dieser Versuchszeit jedoch erst N_{min}-Spuren auf, so dass zwischen ihnen noch keine Unterschiede in der N_{min}-Anhäufung zum Ausdruck kommen. Nach *6 Wochen Bebrütung* (Fig. 2) ergibt sich ebenfalls eine signifikante Korrelation zwischen dem

* N_t = Gesamtstickstoff.

N_t-Gehalt der organischen Substanz und den N_{min}-Werten ($r = 0{,}735$; mit 99,99% Grenzwahrscheinlichkeit gegenüber dem Zufallshöchstwert gesichert). Auch die nach 3 Wochen Versuchsdauer in ihren N_{min}-Quoten noch nicht differenzierten Werte sind jetzt gut zu unterscheiden.

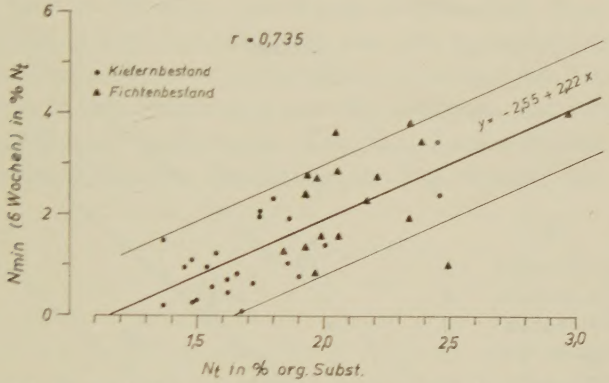

Fig. 2. Beziehung zwischen N_t-Gehalt der organ. Substanz und N_{min}-Anhäufung nach *6 Wochen Bebrütung* bei Rohhumus- und Moderproben.

Bei Wahl einer langen Versuchsdauer verschlechtert sich die Beziehung. Fig. 3 zeigt den Zusammenhang nach *15 Wochen Brutdauer*. Der niedrige Korrelationskoeffizient ($r = 0{,}369$) ist nur noch mit 95% gesichert. Nach dieser langen Brutdauer steigen alle Zeitkurven der N_{min}-Anhäufung etwa linear an, da nun die für Proben mit weitem C : N-Verhältnis typische Anlaufzeit überwunden ist. Jetzt herrschen im bebrüteten Material Verhältnisse, die in keiner Weise mehr den natürlichen Bedingungen entsprechen. Der pH-Wert hat sich durch den angehäuften Mineralstickstoff vom ursprünglichen Wert entfernt, die Wasserkapazität kann sich ebenfalls geändert haben. Infolge des Fehlens der Bodentiere unterbleibt ausserdem die weitere Neuaufbereitung von organischer Substanz. Der Aussagewert der N_{min}-Zahlen nach so langer Bebrütung ist daher gering. Überdies ist der Zeitaufwand für solch langdauernde Brutversuche erheblich. Auch Kaila *et al.* [13] kamen zu dem Ergebnis, dass die Werte nach langer Bebrütung meist schlechtere Beziehungen zum C : N-Verhältnis ergeben als etwa die nach 4 Wochen erhaltenen Zahlen.

Da die Abhängigkeit der N_{min}-Anhäufung von der stofflichen Zusammensetzung in den ersten Wochen der Bebrütung gut zum

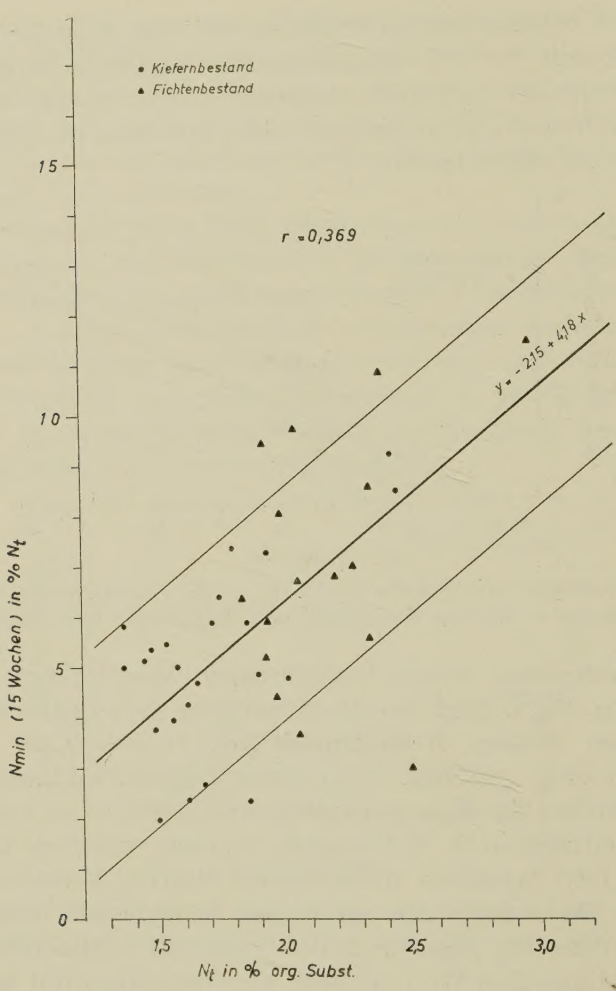

Fig. 3. Beziehung zwischen N_t-Gehalt der organ. Substanz und N_{min}-Anhäufung nach *15 Wochen Bebrütung* bei Rohhumus- und Moderproben.

Ausdruck kommt, empfehlen sich zur Charakterisierung der N_{min}-Anlieferung relativ kurze Brutzeiten. Man darf sie andererseits auch nicht zu knapp wählen, um die N_{min}-Differenzen zwischen sehr N-armen Rohhumusproben noch zu erfassen. *Wir halten daher eine Brutdauer von 6 Wochen bei den von uns eingehaltenen Versuchsbedingungen zur Untersuchung von Waldbodenproben für geeignet.*

12 Beziehung zwischen Stickstoff: Kohlenstoff-Verhältnis und Mineralstickstoff-Anhäufung nach 6 Wochen Bebrütung.

Zur Prüfung des Zusammenhanges zwischen C : N-Verhältnis und N_{min}-Anlieferung bei verschiedenen Waldhumusformen zogen wir die nach 6 Wochen Bebrütung angehäuften N_{min}-Mengen heran. In Fig. 4 sind nun sämtliche von uns untersuchten Proben, also nicht nur die oben zur Feststellung der geeigneten Bebrütungsdauer betrachteten Rohhumus-und Moderproben mit ihren C : N bzw. N_{min}-Werten aufgetragen. Die N_{min}-Zahlen stellen die Anhäufung nach 6 Wochen Bebrütung dar. Fig. 4 zeigt, dass sich die

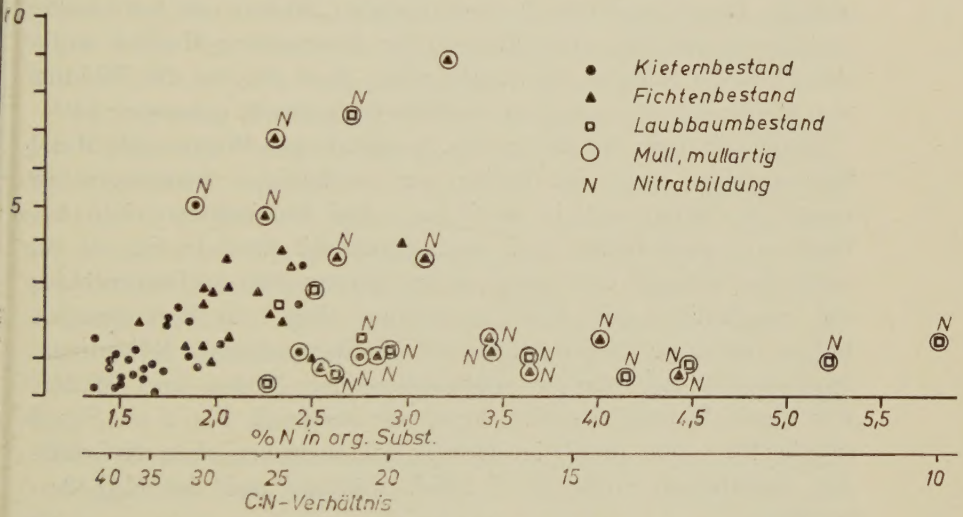

Fig. 4. Beziehung zwischen C : N-Verhältnis und N_{min}-Anhäufung nach 6 Wochen Bebrütung bei verschiedenartigen Humusformen (Messpunkte ohne Ring: Moder und Rohhumus).

C : N-Verhältnisse der untersuchten Proben beinahe über den gesamten, im Boden vorkommenden Bereich erstrecken. Unsere stickstoffreichsten Proben (Laubwaldmull) stossen mit C : N-Werten von 10 in den Bereich des Schwarzerdehumus (Laatsch [15], S. 218) vor. Auf der anderen Seite erreichen die Rohhumusproben extrem hohe, über 40 liegende C : N-Quotienten. Noch höhere Werte findet man nur in Hochmoorböden (etwa 30 bis 70, siehe [7]).

Betrachten wir die Gesamtheit der in Fig. 4 aufgeführten Punkte, so zeigt sich kein Zusammenhang zwischen dem C : N-Verhältnis

bzw. N-Gehalt der organischen Substanz und der prozentualen N_{min}-Anhäufung nach 6-wöchiger Bebrütung. Besonders bemerkenswert ist, dass die Mehrzahl der in der organischen Substanz teilweise enorm N-reichen Mullproben nach 6 Wochen Brutdauer ebenso wie zahlreiche N-arme Rohhumusproben nur bis zu 2% des N_t-Vorrats als N_{min} anhäufen. *Es ist also kein durchgehender Unterschied in der Höhe des N_{min}-Prozentsatzes zwischen Mull- und Rohhumusproben zu erkennen* (vgl. jedoch [36]). Fig. 4 verdeutlicht ferner, dass der organische Stickstoff im Mull und in mullartigen Humusformen fast durchweg nitrifiziert wird (Zeichen N beim Messpunkt), während in Rohhumusproben nur Ammonifikation abläuft. Dass ausserdem die untersuchten Proben von Laubwaldstandorten ziemlich ausschliesslich zur Humusform Mull bis mullartiger Moder gehören, überrascht nicht, da in Bayern die Bildung von Rohhumus bevorzugt an Nadelbaumbestände gebunden ist.

Eliminiert man aus den in Fig. 4 enthaltenen Werten alle Mullhumusproben sowie die Proben aus mullartigen Moderlagen, in denen die Streu rasch in die F-Lage und den humusreichen A_1-Horizont eingearbeitet wird (Signaturen *mit Kreis* in Fig. 4), so verbleiben lediglich die Proben, die wir bereits oben zur Besprechung der zweckmässigsten Bebrütungsdauer (Fig. 1–3) herangezogen haben. Alle diese Proben gehören zu den Humusformen Rohhumus, moderartiger Rohhumus, rohhumusartiger Moder und Moder. Für dieses Material besteht, wenn wir nochmals Fig. 2 mit Fig. 4 vergleichen, eine deutliche Korrelation zwischen dem N-Gehalt der organischen Substanz (C : N-Verhältnis) und der N_{min}-Anhäufung.

Im Rohhumus und Moder (alle Proben sind unter Ausschluss frischer Streu gezogen) *ist der als Mineralstickstoff angehäufte N-Prozentsatz also umso grösser, je enger das C : N-Verhältnis ist* oder mit anderen Worten, je höher der N_t-Gehalt der organischen Substanz liegt. Es wird nicht nur eine dem höheren N-Gehalt der organischen Substanz entsprechend grössere N_{min}-Menge gebildet, sondern eine noch darüber hinaus gehende Steigerung der Mineralstickstoff-Nachlieferung bewirkt.

Die untersuchten Mullproben häufen entweder relativ hohe oder nur geringe N_{min}-Mengen an. Auffällig sind vor allem die wenigen Mullproben, deren N_{min}-Nachlieferung weit über den Werten der Moder- und Rohhumusproben liegt. Eine Prüfung der Humusform

ergab, dass die Proben der Mullgruppe mit hohem N_{min}-Prozentsatz durchweg moderartigen Mull darstellen, in dem bei der Probenahme die sonst einheitlich unberücksichtigte obere Streulage nur schlecht vollständig zu entfernen war. Diese Proben enthalten daher einen gewissen Anteil erst schwach zersetzten Streumaterials. Es weist noch viele leicht angreifbare, N-haltige Stoffe auf, weshalb sich hier die N_{min}-Anhäufung im Gegensatz zu reinem Mullhumus des A_1-Horizonts erhöht *.

Innerhalb der Mullproben von Fig. 4 mit geringer N_{min}-Anhäufung ist keine Korrelation der N_{min}-Werte zum C : N-Verhältnis ersichtlich. Alle diese Proben bestehen zweifellos zum grossen Teil aus echten und sehr stabilen Humusstoffen und unterliegen daher nur schwer und langsam der Mineralisation („Dauerhumus", Scheffer u. Schachtschabel [22], S. 72).

Welch grosser Unterschied in der chemischen Zusammensetzung der organischen Substanz solcher Proben und von Material aus Rohhumuslagen besteht, geht u.a. aus der Untersuchung von Springer u. Klee [24] hervor, deren Untersuchungsmaterial mit den von uns auf ihre N-Mineralisation untersuchten Proben teilweise identisch ist. Vor allem ist der Anteil des leichter mineralisierbaren Amino-N am Gesamt-N bei Mullhumus bedeutend geringer als im Rohhumus oder Moder, wo er häufig über 50% beträgt [31 24]. Die geringen N_{min}-Quoten der Mullproben sind also verständlich.

Man könnte auch für die Mullproben eine Beziehung zwischen C : N-Quotient und N_{min}-Prozentsatz erwarten. Sehr wahrscheinlich ist das vorliegende Material aber trotz grober, humusmorphologisch gleicher Einstufung in Bezug auf seine mikrobielle Angreifbarkeit doch ziemlich verschieden. Die Proben mit relativ niedrigem N-Gehalt dürften erst in geringem Umfang echte Huminstoffe in Ton-Humus-Koppelung aufweisen. Je N-reicher die organische Substanz solcher Proben wird, desto höher ist vermutlich ihr Gehalt an stabilen Humusstoffen und desto geringer ihr mikrobiell leicht zersetzbarer Anteil. Diese Gegenläufigkeit von N-Gehalt der organischen Substanz und mikrobieller Angreifbarkeit überdeckt offenbar die Abhängigkeit der N_{min}-Quote vom C : N-Verhältnis.

* Auf Standorten mit Mullzustand zersetzt sich die fallende Streu i.d.R. binnen eines Jahres. Eine Abtrennung aufliegender Streu war hier bei der Probenahme stets gut möglich.

Die geschilderten Ergebnisse machen klar, dass bei Betrachtung verschiedener Humusformen mit uneinheitlicher mikrobieller Angreifbarkeit keine Beziehung zwischen C : N-Verhältnis und N_{min}-Anlieferung aufscheinen kann. Nur beim Vergleich von N-haltigen organischen Substanzen mit ähnlicher mikrobieller Verwertbarkeit lässt sich der Einfluss des C : N-Quotienten auf die Mineralisation erkennen.

Zu Beginn dieses Abschnitts erörterten wir an Hand der Literatur die Abhängigkeit der N_{min}-Anhäufung vom C : N-Verhältnis des Materials. Die dort genannten Zahlen gelten jedoch nur für leicht zersetzbare organische Substanzen. Aus den Ergebnissen unserer Brutversuche geht hervor, dass keine Übertragung dieser in der Landwirtschaft gebräuchlichen Grenzwerte (bei C : N-Quotienten über 20 bis 25 keine N_{min}-Anlieferung) auf die Verhältnisse der Waldstandorte möglich ist. Nach landwirtschaftlichen Vorstellungen wäre eine N_{min}-Anhäufung in Rohhumus mit C : N-Werten über 40 undenkbar. Sie kann in unseren Waldbodenproben nur ablaufen, weil der grösste Teil der organischen Substanz im Rohhumus mikrobiell äusserst schwer angreifbar (hoher Ligningehalt usf.!) und daher praktisch ohne Einfluss auf das Mineralisationsgeschehen ist.

Die von uns untersuchten Moder- und Rohhumusproben oder die mullartigen Humusformen mit gewissem Streuanteil stellen jeweils eine Gruppe dar, in der die mikrobielle Angreifbarkeit näherungsweise einheitlich ist. Nur für solche Probengruppen ist eine Beziehung zwischen C : N-Verhältnis und N_{min}-Anhäufung erkennbar.

2 *Wirkung von Mineralstickstoff-Zusatz auf die Stickstoffmineralisation im organischen Bodenmaterial.*

Wir sahen oben, dass der N_t-Gehalt der organischen Substanz die N_{min}-Nachlieferung in Waldbodenproben stark beeinflusst. Hat nun auch die Zugabe von Mineralstickstoff eine Wirkung auf das Mineralisationsgeschehen?

Es ist denkbar, dass eine N_{min}-Gabe während des Brutversuchs (wenn schon steigende N_{min}-Anhäufung erfolgt) anders wirkt als wenn man N_{min} bereits zu Beginn der Bebrütung (während der Anlaufperiode) anbietet. Befassen wir uns zunächst mit der Erhöhung des N_{min}-Gehalts durch Zugabe während der Bebrütung.

Hesselman [9], Duchaufour *et al.* [4], Stojanovic u. Broadbent [26] und Van Schreven [23] zeigten in solchen Versuchen mit verschiedenartigem Bodenmaterial übereinstimmend, *dass N_{min}-Zusätze während der bereits laufenden N_{min}-Anhäufung keine Wirkung auf die N-Mineralisation haben.* Eigene Versuche brachten das gleiche Ergebnis. Dies ist erklärlich: Im bereits längere Zeit laufenden Brutversuch mit steigender N_{min}-Anhäufung ist Stickstoff kein begrenzender Faktor für die mikrobielle Tätigkeit. In diesem Stadium der Mineralisation ist die Mikroorganismenaktivität vielmehr durch Mangel an angreifbaren C-Quellen beschränkt. Erst bei einer Erhöhung *dieses* Angebots können die Mikroorganismen die angehäuften oder zugesetzten N_{min}-Mengen verwerten. Eine bloße Zugabe von N_{min} muss daher ohne Wirkung bleiben.

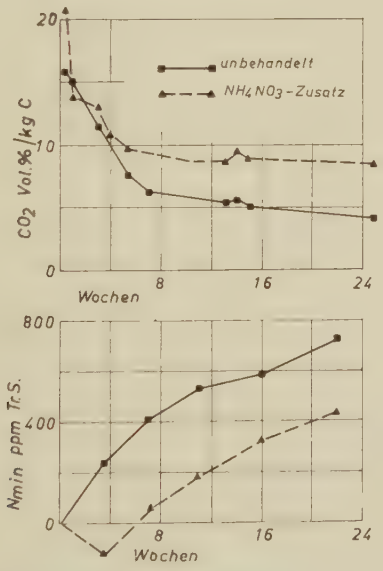

Fig. 5. N_{min}-Anhäufung und CO_2-Produktion von Mull „Ottobeuren" (pH = 3,4; C : N = 20,1). Gestrichelt: Zusatz von je 650 ppm NH_4-N und NO_3N (zugegebener N_{min}-Betrag in der Kurvendarstellung abgezogen).

Gibt man N_{min} bereits bei Beginn der Bebrütung zu, so liegen die Verhältnisse anders. In diesem Fall ist zunächst eine gewisse Immobilisation des zugesetzten N_{min} zu beobachten (vergl. z.B. [19]). Im späteren Verlauf geht die N_{min}-Anhäufung dann ähnlich wie in den unbehandelten Vergleichsproben vor sich. Auch von uns

ausgeführte Versuche zeigten die gleiche Erscheinung (Fig. 5). Dieses Ergebnis ist im Einklang mit dem oben Geäusserten gut zu deuten. *Der schon zu Beginn des Brutversuchs zugesetzte Mineralstickstoff unterliegt teilweise dem sofortigen Verbrauch durch die jetzt in starker Entwicklung begriffene Mikroflora.* Ihr steht nämlich zu diesem Zeitpunkt noch viel verwertbares Kohlenstoffmaterial, aber nur wenig Stickstoff zur Verfügung.

21 Wirkung von Nitratzugabe auf die Stickstoffmineralisation in nicht nitrifizierenden Rohhumus- und Moderproben.

In unseren Modellversuchen zeigten sich bei Zusatz von NH_4- und NO_3-N zu nicht nitrifizierendem Rohhumus und Moder Wirkungen, die von dem im vorhergehend Geschilderten abweichen. Wie aus Fig. 6 hervorgeht, sinken die CO_2-Werte in solchen Proben nach Zugabe von Ammoniumnitrat tief unter die Kurve des Vergleichsmaterials ab. Die zugesetzte NO_3-N-Menge bleibt den ganzen Versuch hindurch erhalten (vgl. auch Fig. 3 in [34]). Die NH_4-N-Kurve steigt in ähnlicher Weise an wie bei der Vergleichsprobe.

Da durch Ammoniumsalze (ohne Nitrat) niemals eine solche starke Erniedrigung der CO_2-Produktion erzielt wurde, kann diese Wirkung nur eine Folge des NO_3-N sein. Welcher Art ist nun diese Beeinflussung? Der pH-Wert ändert sich durch diese Stickstoffsalzgabe nicht. Eine Beeinträchtigung der mikrobiellen Tätigkeit infolge zu starker Salzkonzentration ist bei den gegebenen Mengen ebenfalls nicht anzunehmen, denn in langdauernden Brutversuchen stellt man in nicht nitrifizierenden Rohhumusproben noch wesentlich höhere N_{min}-Gehalte (weit über 1000 ppm) fest, ohne dass ein Einfluss auf das Mineralisationsgeschehen erkennbar ist (vgl. [33] oder [18]). Es muss sich also um eine Giftwirkung des NO_3-N auf spezifische Rohhumusbewohner handeln. Sie kann jedoch nur einen Teil der Mikroorganismen betreffen, da zwar die CO_2-Produktion, nicht aber die Anhäufung von NH_4-N beeinträchtigt wurde.

Zur weiteren Prüfung des Nitrateinflusses führten wir einen Versuch mit steigenden NO_3-N-Gaben durch. Als Versuchsmaterial diente ein ebenfalls nicht nitrifizierender, moderartiger Rohhumus (Fig. 7). Die gewählten Salzmengen, die wir auch hier als wässerige

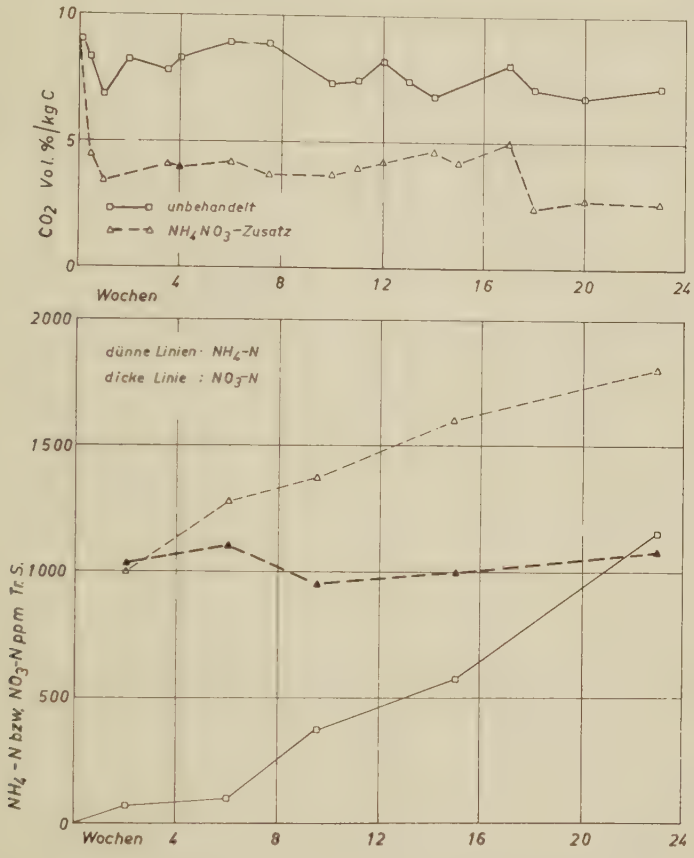

Fig. 6. N_{min}-Anhäufung und CO_2-Produktion von Rohhumus „Amberg" (pH = 2,76; C : N = 30,5) ohne und mit Zusatz von je 1000 ppm NH_4-N und NO_3-N (als NH_4NO_3).

Lösung zumischten, gehen aus der Legende zu Fig. 7 hervor. Teilweise setzten wir gleich hohe NH_4-N- und NO_3-N-Mengen in Form von Ammonnitrat und als K- bzw. P-Salze * an,

Das Ergebnis ist eine deutliche Staffelung der CO_2-Kurven in Abhängigkeit von der Höhe der NO_3-Gaben (Fig. 7). Je grösser die NO_3-Mengen sind, umso stärker wird die CO_2-Produktion gehemmt. Dabei spielt es keine Rolle, ob NO_3-N allein oder in Verbindung

* Die K- und Phosphationen bliebe n offenbar ohne Einfluss auf das Mineralisationsgeschehen.

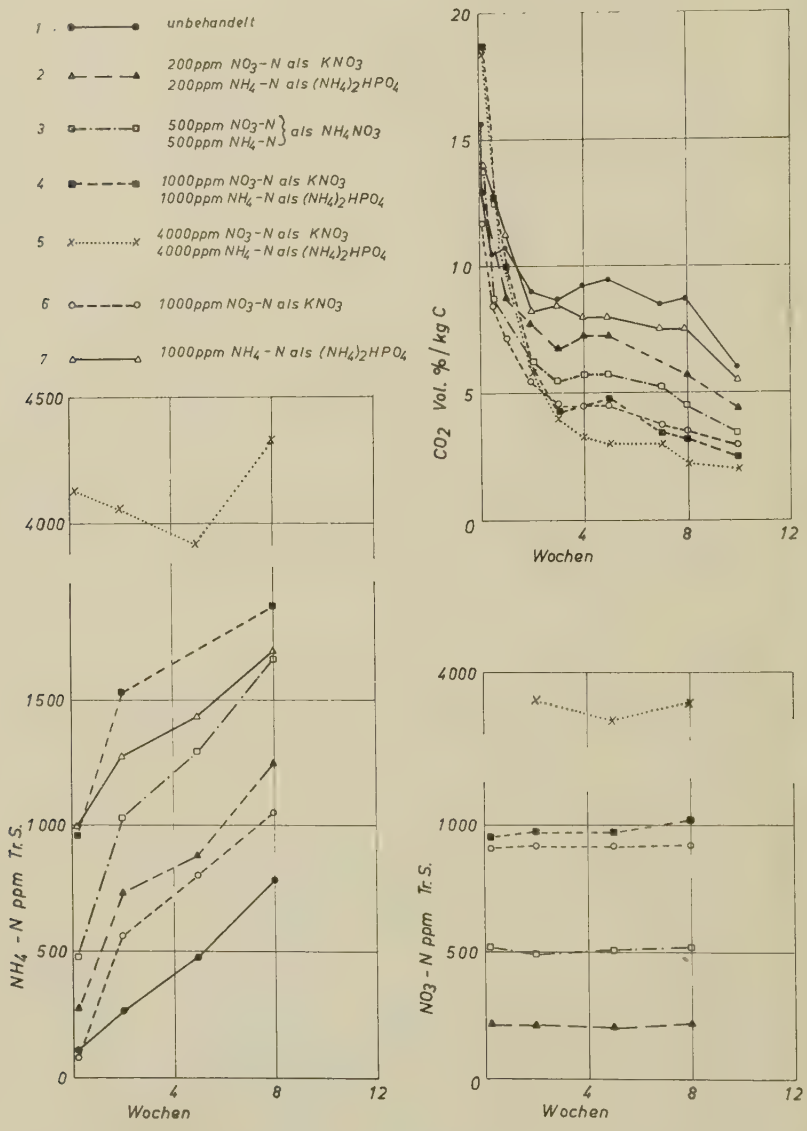

Fig. 7. NH_4-N-Anhäufung, NO_3-N-Gehalt und CO_2-Produktion bei der Bebrütung von Rohhumus „München-Süd" (pH = 2,95; C : N = 25,7) nach Zusatz gestaffelter NO_3-N- bezw. NH_4-N-Gaben.

mit NH_4-N gegeben wurde (ähnliche Kurven der Versuchsglieder 4 und 6 in Fig. 7!). Die gegebenen Nitratmengen wurden erstmals am Tag nach dem Zusatz bestimmt (Ausgangswerte in Fig. 7). Sie finden sich den Versuch hindurch mit nur geringer Streuung stets in der gleichen Höhe wieder. Die NH_4-N-Anhäufung geht in allen Versuchsgliedern unvermindert weiter, wobei die mit NO_3-N behandelten Proben sogar einen steileren Anstieg der NH_4-N-Kurven zeigen als die Serien ohne Nitratzusatz. Dadurch haben die Versuchsglieder 2, 3, 4 und 6 (*mit* NO_3-Zusatz) * nach 2 bzw. 5 Wochen Bebrütung im Durchschnitt bereits 410 bzw. 720 ppm NH_4-N angehäuft, während die Versuchsglieder 1 und 7 (*ohne* NO_3-Zusatz) nur 220 bzw. 515 ppm NH_4-N enthalten.

Auch bei anderen, nicht nitrifizierenden Rohhumusproben, die wir untersuchten, beeinflussten Nitratgaben die CO_2-Produktion und NH_4-N-Anhäufung in gleicher Weise. Die in Fig. 7 dargestellten Ergebnisse lassen sich daher in gewissem Umfang verallgemeinern: *Bei nicht nitrifizierenden Rohhumusproben erniedrigt Nitratzusatz die CO_2-Produktion und erhöht die NH_4-N-Anhäufung.*

Wir deuten diesen „Nitrat-Effekt" als Giftwirkung auf einen Teil der Mikroorganismen. Da die NO_3-N-Mengen während der Versuchsdauer in gleicher Höhe nachweisbar bleiben, muss die Schädigung der Mikroorganismen ohne merkliche Aufnahme von NO_3 eintreten. Möglicherweise kommt die Wirkung durch in Spuren gebildetes Nitrit zustande. Es ist aber auch folgende Erklärung möglich: Durch wasserlösliche, stark reduzierende organische Stoffe wird im Rohhumus ein niedriges Redoxpotential erzeugt. Die Mikroflora des Rohhumus ist daran angepasst. Gibt man nun Nitrat hinzu, so erhöht sich der rH-Wert entsprechend, was eine Reihe von Mikroorganismen nicht vertragen. In nitrifizierenden Mullproben herrschen von vorneherein höhere rH-Werte; die hier lebende Mikrobenpopulation ist durch Nitratgaben daher nicht zu schädigen.

Die NO_3-Wirkung setzt (siehe CO_2-Kurve in Fig. 6) unmittelbar nach Beginn der Bebrütung noch nicht ein. Wie weiter oben ge-

* Die mit je 4000 ppm NH_4-N und NO_3-N versetzten Proben (Versuchsglied 5) zeigen die stärkste CO_2-Hemmung und keine vermehrte NH_4-N-Anhäufung. Durch solch übermässig hohe Gaben, die auch im lange bebrüteten Humus nicht erreicht werden, ist offenbar eine gewisse Schockwirkung eingetreten. Dieses Versuchsglied ist deshalb in diesen Zahlen nicht berücksichtigt.

schildert, dürfen wir in unseren Rohhumusproben in dieser ersten Zeit vor allem rege Bakterientätigkeit annehmen. Sie erfährt durch Nitrat offenbar keine merkbare Hemmung. Erst mit dem Aufleben der Pilzaktivität (Anstieg zum 2. Gipfel der CO_2-Kurve) zeigt sich der Nitrat-Effekt deutlich. Teilweise ist in den CO_2-Kurven bei Nitratzusatz gar kein zweiter Gipfel mehr zu erkennen. Dieses Verhalten legt die Vermutung nahe, dass NO_3-N in erster Linie auf die Pilzflora des Rohhumus schädigend wirkt. Die verstärkte NH_4-N-Anhäufung in den nitratbeeinflussten Proben kann man ebenfalls durch eine relative Unterdrückung der Pilztätigkeit bei gleichzeitig ungehemmter Bakterienaktivität erklären: Eine vorwiegend von Bakterien ohne Konkurrenz der Pilze getragene N-Mineralisation führt schon bei relativ geringem Bruttoumsatz zu hoher N_{min}-Anhäufung, da der „ökonomische Koeffizient" * bei Bakterien nach Mikola [17] höchstens 10% beträgt. Pilze arbeiten hingegen mit einem wesentlich höheren Koeffizienten von 20 bis 30% des Trockensubstanzverlustes. Sie verbrauchen also bei der Mineralisation einer bestimmten Menge N-haltiger organischer Substanz mehr Stickstoff zum Aufbau eigener Körpersubstanz als die Bakterien. Dadurch verringert sich die Mineralstickstoff-Nachlieferung entsprechend. Wird nun – wie wir für unsere Versuche annehmen – die Lebenstätigkeit der Pilze gehemmt, so überwiegt die Mineralisationsarbeit der Bakteriengruppen. Infolge ihrer geringeren Immobilisationsquote erfolgt eine höhere N_{min}-Anlieferung.

Die Mikrobiologie der Käserei- und Gärungstechnik liefert ebenfalls Anhaltspunkte für unsere Annahme. So wird im Käsereibetrieb Nitrat zugesetzt, um unerwünschte Gasbildung durch anaerobe Bakterien oder Fremdinfektionen von Pilzen zu verhindern. Jörgensen u. Hansen [12] erwähnen die Giftwirkung von Nitrat auf Brauereihefe. Die Hemmwirkung tritt hier schon bei geringeren Nitratkonzentrationen auf als die Bodenlösung in unseren Brutversuchen enthält. Mit der Schädigung der Hefe setzt dann steigende Bakterieninfektion ein.

Wir dürfen somit sagen: *Ein Teil der im Rohhumus lebenden Mikroorganismen wird durch Nitrat gehemmt. Die beobachtete Steigerung der NH_4-N-Anhäufung könnte auf eine stärkere Schädigung der Pilzflora zurückzuführen sein.* Andererseits wäre durch die

* Der „ökonomische Koeffizient" drückt die beim Abbau organischer Substanz wieder immobilisierte Menge in Prozent des Trockensubstanzverlustes aus.

Giftwirkung des Nitrats aber auch eine Teilsterilisation mit anschliessender Autolyse denkbar. In Humusformen mit Nitrifikation ist kein „Nitrat-Effekt" zu erwarten *, da in solchem Material die Tätigkeit der Bakterien überwiegt und die vorkommenden Pilzgruppen an das laufend gebildete Nitrat bzw. hohe rH-Werte angepasst sind.

Wie die in Fig. 6 bei Zöttl [37] dargestellten Ergebnisse zeigen, tritt der „Nitrat-Effekt" bei extremer biologischer N-Sperre offenbar nicht mehr auf. In solchen Fällen (wir fanden das gleiche Ergebnis auch in weiteren unveröffentlichten Versuchen, bei denen wir in nicht nitrifizierenden Proben durch Saccharose-Zusatz eine N-Sperre hervorriefen) ist keine Hemmwirkung des Nitrats erkennbar, hingegen aber deutlich seine Immobilisation.

3 *Bedeutung von Huminstoffgehalt und Kohlenstoff: Stickstoff-Verhältnis des Bodenmaterials für die Stickstoffmineralisation.*

In den vorhergehenden Abschnitten sahen wir, dass die mikrobielle Angreifbarkeit des organischen Bodenmaterials und ihr N-Gehalt die Mineralstickstoff-Anhäufung stark beeinflussen. Wir erhielten Hinweise, dass die mikrobielle Verwertbarkeit der organischen Substanz bedeutsamer für die N_{min}-Anlieferung ist als ihr N-Gehalt. Dies zeigte uns vor allem auch die Betrachtung der Beziehung zwischen C : N-Verhältnis und Mineralisationsquote bei verschiedenen Humusformen. Dabei ergaben sich für Proben mit geringem Anteil schwach zersetzten Pflanzenmaterials und hohem Gehalt stabiler Huminstoffe (= Mullhumus) trotz engem C : N-Verhältnis nur sehr geringe Werte der N_{min}-Anlieferung. Diese Proben unterscheiden sich jedoch nicht nur durch geringere mikrobielle Angreifbarkeit, sondern auch noch hinsichtlich vieler anderer massgebender Eigenschaften von Moder- oder Rohhumusproben. Wir versuchten daher die Frage nach der Prävalenz des Faktors „mikrobielle Zersetzbarkeit" oder des Faktors „C : N-Verhältnis" durch Untersuchungen am gleichen Humusmaterial bzw. an Humuslagen des gleichen Standorts zu klären.

Zwei Wege wurden beschritten. Einmal prüften wir verschiedene Siebfraktionen des gleichen Rohhumusmaterials auf ihre N-Mineralisation. Es war zu erwarten, dass sich in den feiner gesiebten

* Dies bestätigen unveröffentlichte Versuche.

Proben Humusstoffe und schwerer angreifbare Gewebebestandteile anreichern. Diese Fraktionen müssten ein engeres C : N-Verhältnis als die gröberen Anteile aufweisen. Zum anderen entnahmen wir auf einigen Standorten die einzelnen Lagen von Rohhumus (und Moder) getrennt zur Bebrütung. In den oberen Lagen, vor allem in der L-Lage, befinden sich noch viele und leicht verwertbare pflanzliche Gewebestücke mit hohem C : N-Verhältnis, während in den tieferen Lagen (vor allem im A_1-Horizont) stabile Humusstoffe mit niedrigerem C : N-Quotienten angereichert sind. Sowohl in den verschiedenen Siebfraktionen als auch in den einzelnen Lagen des Humushorizonts liegt ein relativ einheitliches Material vor, denn auch die verschiedenen Humuslagen sind ja grossenteils aus der gleichen Streu hervorgegangen. Dieses Versuchsmaterial unterscheidet sich hauptsächlich durch die Gegenläufigkeit von C : N-Verhältnis und Prozentsatz leicht angreifbarer organischer Substanzen.

Wir gaben eine Reihe naturfeuchter Moder- und Rohhumusproben durch Siebe verschiedener Maschenweite (1 bis 7 mm). Trotz deutlicher morphologischer Unterschiede in der Zusammensetzung der Fraktionen waren ihre C : N-Zahlen jedoch annähernd gleich. Auch der Mineralisationsablauf im Brutversuch differierte kaum. Die Untersuchung der Siebfraktionen ergibt daher keine Antwort auf unsere Frage.

Hingegen unterschieden sich die einzelnen Humuslagen der von uns untersuchten Moder- und Rohhumusproben deutlich im C : N-Verhältnis und Gehalt an Huminstoffen. Wie aus der Legende von Fig. 8 und 9, in denen die Ergebnisse zweier Versuche als Beispiele dargestellt sind, hervorgeht, nimmt der C : N-Quotient von der L-Lage über F- und H-Lage zum Humus des A_1-Horizontes deutlich ab. Die Anreicherung stabiler Humusstoffe dürfte hingegen in der Streu (L-Lage) am geringsten, im A_1-Horizont am grössten sein. Dies zeigen die Analysenwerte von „Podsol Amberg" bei Springer u. Klee [24], die am gleichen Material gewonnen wurden, wie es im Brutversuch „Rohhumus Amberg", Fig. 8, Verwendung fand.

Beim Kiefern-Rohhumus „Amberg" (Fig. 8) und im Fichten-Rohhumus „Sachsenried" (Fig. 9) zeigen CO_2-Produktion und N_{min}-Anhäufung eine übereinstimmende deutliche Staffelung entsprechend den einzelnen Humuslagen. *CO_2- und N_{min}-Werte liegen um so tiefer, je stärker die Lagen zersetzt und mit stabilen Humus-*

stoffen angereichert sind – trotzdem das C : N-Verhältnis in diesen tieferen Humuslagen bedeutend enger ist. Es überrascht, dass trotz des erwarteten hohen Bruttoumsatzes in der schwach zersetzten und mikrobiell leicht angreifbaren Streu (F-Lage) auch die höchste N_{min}-Anhäufung festzustellen ist. Die stabilen Huminstoffe der

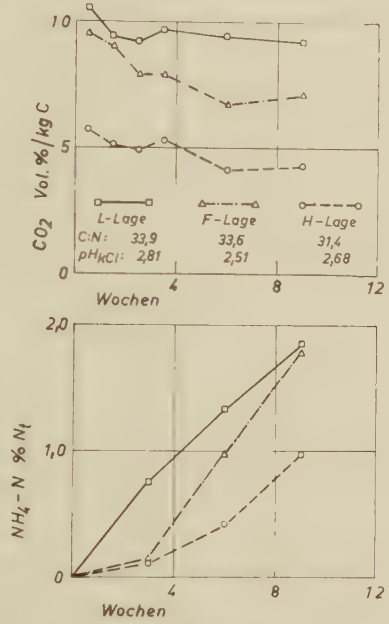

Fig. 8. N_{min}-Anhäufung und CO_2-Produktion von L-, F- und H-Lage des Rohhumus „Amberg".

H-Lage und des an kolloidalem Humus reichen A_1-Horizonts verhalten sich wie Mullhumusformen: Ungeachtet des höheren N_t-Anteils in der organischen Substanz liefern sie die geringsten N_{min}-Werte. Die schlechte mikrobielle Angreifbarkeit ist wohl die Ursache hierfür.

Diese Ergebnisse decken sich mit Befunden von Krauss u. Prescher [14], die an Hand von CO_2-Messungen ebenfalls eine Abnahme der Mineralisationsintensität von der L- zur H-Lage feststellten. Duchaufour [3] erhielt für die N_{min}-Anlieferung der einzelnen Humuslagen des gleichen Standorts mit unseren Resultaten übereinstimmende Tendenzen. Leider hatten die genannten Verfasser jeweils nur die N_{min}-Anhäufung *oder* die Brutto-Minera-

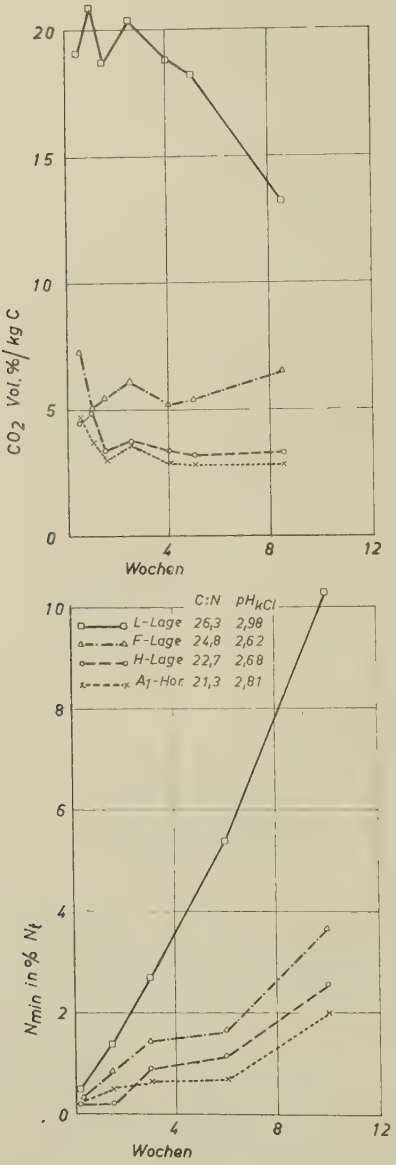

Fig. 9. N_{min}-Anhäufung und CO_2-Produktion der Humuslagen des moderartigen Rohhumus „Sachsenried".

lisation allein gemessen und nicht, wie wir, beide Grössen am gleichen Material untersucht.

Ein abweichendes Verhalten schildert Fenton [6]. Er verglich in Brutversuchen mit Perkolationstechnik [16] die Netto-Mineralisation von gemahlener Birken- und Kiefernstreu allein und in Mischung mit Mullboden. Die eigemischte Kiefernstreu drückte dabei die N_{min}-Anhäufung im Mullboden stark herab, was auf hohe Brutto-Mineralisation bei geringer Netto-Anlieferung schliessen lässt. Im Gegensatz zu unseren Versuchen arbeitete Fenton mit *frischer* Streu, die einen hohen Anteil leicht verwertbarer organischer Substanzen enthalten dürfte. Unsere L-Lagen-Proben umfassen hingegen nur Streu, die älter als 1 Jahr ist. Zudem wurden sie nicht gemahlen. Die Daten Fentons machen jedenfalls wahrscheinlich, dass die frisch gefallene Streu zunächst keine gute N_{min}-Quelle darstellt.

Weitere, von uns mit Laubwald-Moderlagen durchgeführte Brutversuche zeigten ebenfalls die gleiche Staffelung der Werte, wie wir sie oben für Rohhumusproben schilderten. *Alle Brutversuche mit Material der einzelnen Humuslagen ergeben somit übereinstimmend, dass der Faktor „mikrobielle Angreifbarkeit" die Bedeutung des C : N-Verhältnisses für die Anhäufung von N_{min} überlagert* *. Dies steht in Einklang mit den früher geschilderten Untersuchungen über die Abhängigkeit der N_{min}-Anhäufung von der Menge verfügbaren N-freien C-Materials [37] und deckt sich mit den an Ackerböden unter Verwendung von N^{15}-Technik erzielten Ergebnissen von Jansson [10].

ZUSAMMENFASSUNG

Der Einfluß des Stickstoffgehaltes auf die Mineralstickstoff-Nachlieferung wird in Laborbrutversuchen mit Waldhumusmaterial geprüft. Es zeigt sich eine gewisse Abhängigkeit der Mineralstickstoff-Nachlieferung vom Gesamtstickstoffgehalt des organischen Materials (C : N-Verhältnis).

Der Faktor „mikrobielle Zersetzbarkeit" überlagert jedoch diese Beziehung. Daher kann man aus dem C : N-Verhältnis keine allgemein gültigen Schlüsse auf die Mineralstickstoff-Nachlieferung ziehen. Nur beim Vergleich von Material mit ähnlicher mikrobieller Zersetzbarkeit besteht eine enge Abhängigkeit zwischen den beiden Größen. Eine solche Beziehung ist für die

* Allerdings darf nicht unberücksichtigt bleiben, dass die pH-Werte in den weniger abgebauten Humuslagen meist etwas höher liegen und auch aus diesem Grunde eine bessere Mineralisation erfolgen kann (vgl. Zöttl [38]).

untersuchten Rohhumus- und Moderproben zu erkennen. Bei diesem Humusmaterial liegt die Mineralisationsquote um so höher, je enger das C : N-Verhältnis ist. Die Korrelation ist nach 6 Wochen Brutversuch besser als nach 3 oder 15 Wochen.

Für Mullhumus und für Humus aus der Humusstofflage sowie dem A_1-Horizont von Bodenprofilen mit organischer Auflage ergeben sich trotz engem C : N-Verhältnis meist nur sehr geringe Mineralisationsquoten. Die Zahlen liegen ähnlich niedrig wie bei ungünstigen Rohhumusformen mit weitem C : N-Verhältnis. Ursache hierfür dürfte der hohe Gehalt dieser Humusformen an stabilen Huminstoffen sein. Proben aus der F-Lage von Moder haben hingegen häufig hohe Mineralisationsquoten. Die höchsten Werte von Brutto- *und* Nettomineralisation treten bei Material aus der Streulage auf.

Mineralstickstoff-Zusätze während der Bebrütung bleiben ohne Einfluß auf den Mineralisationsablauf – außer man gibt Nitrat zu nicht nitrifizierenden Rohhumus-Proben. In diesem Fall ist eine Hemmwirkung auf einen Teil der Mikroflora zu beobachten. Die Kohlendioxyd-Produktion sinkt, die Mineralstickstoff-Anhäufung ist jedoch erhöht. Zu Beginn der Bebrütung zugegebener Mineralstickstoff unterliegt der Immobilisation.

SUMMARY

The influence of the nitrogen content on the mineral-nitrogen supply was investigated in laboratory incubation studies with humus material of forests. It shows a certain dependence of the mineral-nitrogen supply on the total nitrogen content of the organic matter (C : N ratio).

However, the factor "microbial decomposability" directly influences this correlation. Therefore one cannot draw generally valid conclusions of the mineral-nitrogen supply from the C : N ratio. Only when comparing material with similar microbial decomposability is there a close correlation between both the quantities. Such a relation is seen for the raw-humus and moss-humus samples which were tested. In this humus material the higher the mineralization rate the lower is the C : N ratio. The correlation after 6 weeks incubation is closer than after 3 or 15 weeks.

Mull humus, and also humus from the H-layer and from the A_1-horizon of raw-humus profiles generally show very low mineralization rates in spite of their low C : N ratio. These mineralization rates are mostly as low as the rates for poor raw-humus samples with high C : N ratio. The cause is the high content of stable humin substances. In contrast samples from the F-layer of moss humus often have high mineralization rates. The highest values of gross- *and* net-mineralization appear in material from the L-layer.

Addition of mineral-nitrogen during the incubation has no influence on the mineralization of organic nitrogen – except for the addition of nitrate to non-nitrifying raw-humus samples. In this case a check effect upon a part of the microflora is observed. The CO_2 production decreases, but the mineral-nitrogen accumulation increases. At the beginning of incubation, added mineral-nitrogen is immobilized.

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DYNAMIK DER STICKSTOFFMINERALISATION IM ORGANISCHEN WALDBODENMATERIAL

III. pH-WERT UND MINERALSTICKSTOFF-NACHLIEFERUNG

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EINLEITUNG

Ein Einfluss des pH-Wertes auf die Stickstoffmineralisation ist seit langem bekannt und vielfach untersucht (vergl. die Zusammenstellung von Harmsen u. van Schreven [9]). Besonders die Abhängigkeit der Nitratbildung vom pH-Wert wurde häufig behandelt. Bei Waldstandorten ist ein weitgehender Zusammenhang zwischen hohen pH-Werten und guter Mineralstickstoff-Nachlieferung erkennbar; in stark sauren Böden stockt die Mineralisation meistens (Hesselman [13 14], Aaltonen [1], Tamm [28], Wittich [36]). Nitrifikation ist in der Regel an pH-Werte über 3,5 (in Wasser gemessen) gebunden; Ausnahmen sind jedoch nicht selten.

Wir prüften, ob die aus zahlreichen Laborbrutversuchen (Zöttl [42 43 45]) vorliegenden Zahlen auch derartige Beziehungen erkennen lassen. Weiterhin untersuchten wir die Veränderungen des pH-Werts, die als Folge der N_{min}-Anhäufung in bebrütetem Material auftreten. Ausserdem wollten wir an Hand von Modellversuchen die Frage beantworten, inwieweit eine Erhöhung des pH-Werts durch Kalziumkarbonat-Zusatz die Stickstoffmineralisation in verschiedenen Waldhumusproben von Standorten Bayerns beeinflusst.

VERSUCHSMATERIAL UND METHODIK

Die untersuchten Proben sind aus dem A_0-Horizont (meist F-Lage) oder dem obersten A_1-Horizont von verschiedenen Standorten im Staatswald Bayerns entnommen. Eine genaue Schilderung der Probenahme gaben wir

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bereits früher an anderer Stelle [42]. Im übrigen enthalten die Figuren-Unterschriften z.T. weitere Daten.

Die von uns angewandte Brutversuchsmethodik wurde bereits ausführlich beschrieben [41 42 44]. Gleiches gilt für die chemischen Analysenmethoden [42 44]. Alle angegebenen pH-Werte sind in 1 *N* KCl-Lösung gemessen.

ERGEBNISSE UND DISKUSSION

1 *Beziehung zwischen dem pH-Wert vor der Bebrütung und der Mineralstickstoff-Anhäufung im Brutversuch.*

Tragen wir die im Brutversuch in 66 Proben verschiedener Humusform gemessene N_{min}-Anhäufung * in Prozent des N_t * der einzelnen Proben gegen ihre zu Beginn der Bebrütung gemessenen pH-Werte auf, so ergibt sich *kein* durchgehender Zusammenhang (Fig. 1). Die Mullproben erreichen zwar die höchsten pH-Zahlen, zeigen aber eher eine nicht lineare, negative Beziehung zwischen pH-Wert ** und N_{min}-Anhäufung. Auf Grund ihres hohen Gehalts schwer angreifbarer Huminstoffe häuft sich bei ihnen, wie bereits

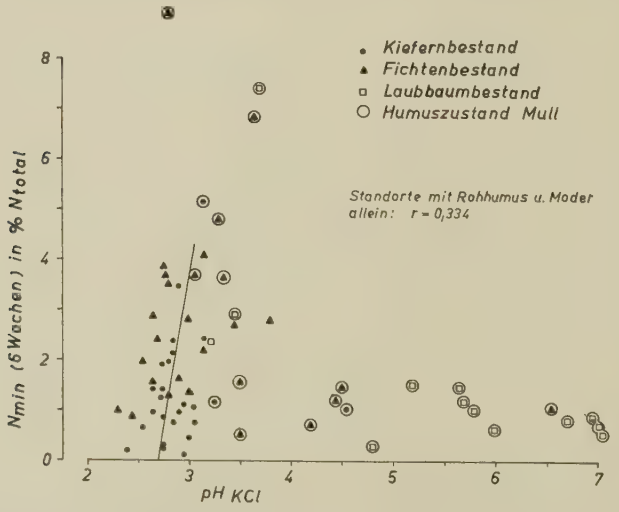

Fig. 1. Beziehung zwischen pH-Wert (vor der Bebrütung) und N_{min}-Anhäufung nach 6 Wochen Brutversuch bei Proben verschiedener Humusform.

* N_{min} = Mineralstickstoff (NH_4-N *und* NO_3-N);
N_t = Gesamtstickstoff (Kjeldahl).

** Statt des pH-Wertes würde man natürlich richtiger die Wasserstoffionen-Konzentration heranziehen. Die Streuung der Werte verändert sich durch eine derartige Umrechnung jedoch nicht. Wir haben uns deshalb mit den anschaulicheren pH-Werten begnügt.

früher [45] betont, nur ein geringer Prozentsatz des organisch gebundenen Stickstoffs in mineralisierter Form an. Moderartiger Mull, dessen Streulage sich am Standort weniger rasch zersetzt, reagiert bereits sauer. Er liefert aber häufig mehr N_{min} (ausgedrückt in % N_t) an als echter Mull, da in ihm noch mehr mikrobiell leicht verwertbare Substanzen enthalten sind.

Greifen wir aus den in Fig. 1 aufgeführten Werten die Moder- und Rohhumusproben heraus, so lässt sich für sie eine schwache positive Korrelation zwischen pH-Wert und N_{min}-Anhäufung errechnen ($r = 0{,}334$; gegen $P = 5\%$ gesichert). Je niedriger der pH-Wert dieser Humusformen liegt, desto geringer wird die N_{min}-Anlieferung. Die Untersuchungen von Krauss u. Thomas [19] und Krauss u. Gude [20] ergaben für das Tharandter Forstlehrrevier bei Dresden den gleichen Zusammenhang. Der niedrige Korrelationskoeffizient unseres Materials zeigt, dass diese Beziehung bei wenigen Zahlenunterlagen nicht nachzuweisen sein kann.

Zwischen pH-Wert und Humusform besteht ein loser Zusammenhang. Ausserdem sahen wir früher [45], dass auch das C : N-Verhältnis eine gewisse Beziehung zur Humusform aufweist. Es lag deshalb nahe, das vorhandene Zahlenmaterial auf die Korrelation zwischen pH-Wert und C : N-Verhältnis zu prüfen. Diese in Fig. 2

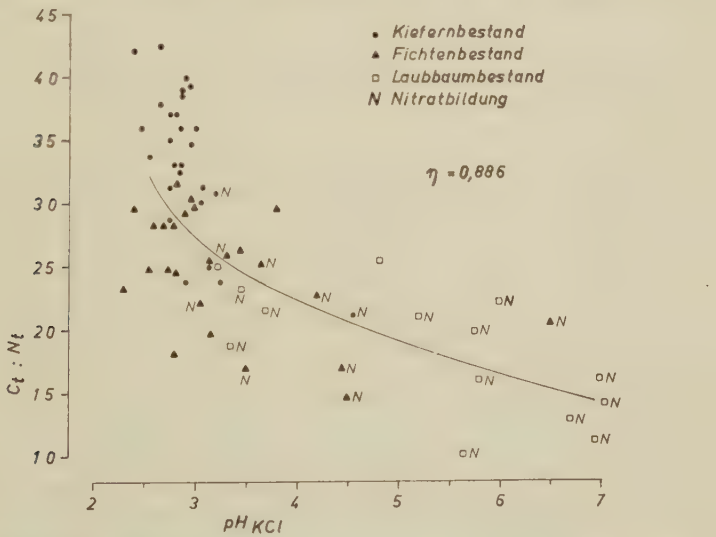

Fig. 2. Beziehung zwischen pH-Wert (vor der Bebrütung) und C : N-Verhältnis von Proben verschiedener Humusform.

dargestellte Beziehung ist bei den von uns untersuchten Waldbodenproben eng und erstreckt sich über alle Humusformen. Der ursächliche Zusammenhang ist klar: Auf basenreichen Böden verläuft die Streuzersetzung durch Mikroorganismen und Bodenfauna rasch und vollständig. Infolge der nur mässig sauren pH-Werte bilden sich in hohem Maße stabile Huminstoffe mit hohem N-Gehalt. Bei basen- und nährelementarmen Böden hingegen liegen die pH-Werte in den entstehenden Humuslagen tief. Der Abbau von anfallender organischer Substanz ist gehemmt. Dies führt zur Anhäufung schwach zersetzten und nur Huminsäurevorstufen enthaltenden organischen Materials. Der N-Gehalt eines solchen, stofflich nur wenig umgewandelten Materials ist niedrig. Er liegt vielfach noch ähnlich tief wie in den abgefallenen Nadeln der Streulage (vgl. Wittich [34] und Viro [31]).

Aus Fig. 2 lässt sich ferner die Abhängigkeit der Nitrifikation vom pH-Wert ablesen. Die untersuchten Waldhumusproben mit pH-Werten über 4 nitrifizieren fast alle (Zeichen N am Messpunkt). Im pH-Bereich 3 bis 4 finden wir schon mehrere lediglich ammonifizierende Proben. Bei pH-Zahlen unter 3,1 zeigt sich keine Nitrifikation mehr. Dieses Ergebnis stimmt mit anderen Beobachtungen überein. Allerdings verschieben unsere Ergebnisse die pH-Grenze der Nitrifikation noch weiter in den sauren Bereich. Nur in Hochmoorböden findet nach Kaila [16] bei so hoher Wasserstoffionenkonzentration gelegentlich noch Nitrifikation statt.

2 *Änderung des pH-Werts im Laufe der Bebrütung*

Während des Brutversuchs ändert sich die Wasserstoffionenkonzentration. In nur ammonifizierendem Bodenmaterial steigen die pH-Zahlen entsprechend den angehäuften NH_4-Mengen stetig an (siehe Zöttl [40]). Bei starker N-Nettomineralisation in schlecht gepufferten Proben kann nach mehreren Wochen Versuchsdauer der pH-Wert sogar um einige Zehntel gegenüber dem Ausgangswert zunehmen.

Wo Nitrifikation abläuft und neben geringen Ammoniummengen vor allem der Gehalt an NO_3-N ansteigt, sinken die pH-Zahlen hingegen deutlich ab (vgl. Scheffer u. Welte [27]). Die Änderung der Bodenreaktion durch den angehäuften Mineralstickstoff wird besonders augenfällig, wenn die Nitrifikation des zunächst angehäuften Ammoniumstickstoffs erst nach einigen Wochen Bebrütung

beginnt. In solchen Proben hört das Steigen der pH-Werte während der Ammonifikationsperiode mit dem Auftreten der ersten Nitratmengen rasch auf und macht einer zunehmenden Versauerung Platz. In Fig. 3 ist ein solches Verhalten am Beispiel des gekalkten Rohhumus „München-Süd" dargestellt.

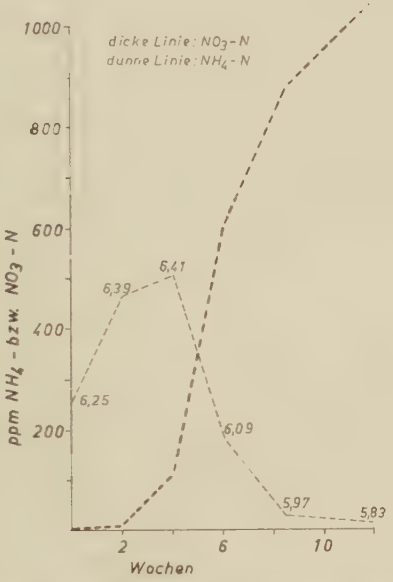

Fig. 3. N_{min}-Anhäufung und pH-Werte (Zahlen an der NH_4-N-Kurve) im Brutversuch mit gekalktem Rohhumus „München-Süd".

Es ist denkbar, dass die in manchen Proben nach mehreren Wochen Bebrütung einsetzende Nitrifikation erst durch die allmähliche Erhöhung des pH-Werts während der NH_4-N-Anhäufung ermöglicht wird.

3 *Wirkung der pH-Erhöhung durch Kalkung auf die Stickstoffmineralisation*

Wir sahen oben, dass die Höhe der N-Mineralisation in gewissem Umfang vom pH-Wert der Proben abhängt. Inwieweit beeinflusst nun eine Veränderung des pH-Wertes infolge Zugabe basisch wirkender Salze die N-Mineralisation? Eine Erhöhung des pH-Wertes ist durch Zusatz von $CaCO_3$ leicht möglich. Auf Grund der vorhergehenden Ausführungen ist als Folge dieser Maßnahme eine grössere N_{min}-Anlieferung zu erwarten.

Auch im Walde durchgeführte Düngungsversuche geben Hinweise zur Beantwortung unserer Frage. Sie zeigen, dass man durch die Kalkung von

Rohhumus, die in erster Linie mit dem Ziel einer Verbesserung der Humusform in Richtung Mull durchgeführt wurde, häufig die Mineralstickstoff-Nachlieferung verstärkt (Wittich [33 35 36 38 39]; Hausser u. Schairer [12]; Hausser [10 11]; Mitscherlich [23]; Duchaufour u. Guinaudeau [5]).

In unseren Versuchen verfolgten wir nun die durch den Zusatz von kohlensaurem Kalk hervorgerufene pH-Erhöhung in ihrer Wirkung auf die N-Mineralisation durch Messung der N_{min}-Anhäufung und CO_2-Produktion *. Wir führten die Kalkungen an Labor-Brutproben aus, da die Feststellung der Umsetzungsvorgänge so besser möglich ist als bei Freilandversuchen. Da je nach Humusform verschiedene Wirkungen zu erwarten waren, verwendeten wir in unseren Modellversuchen Mull-, Moder- und Rohhumusproben.

Zunächst sei ein Kalkungsversuch mit Mull „Ottobeuren"

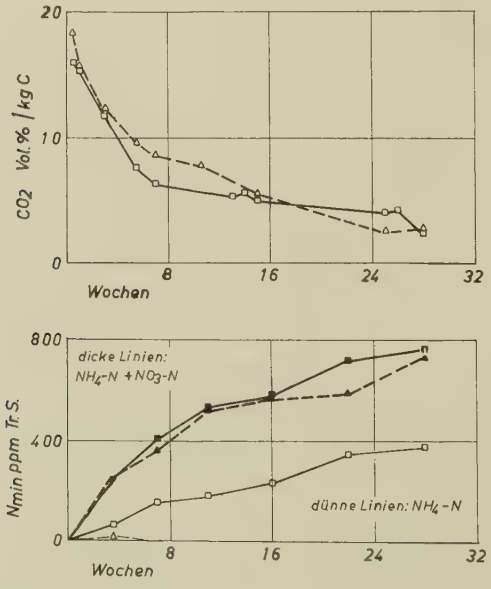

Fig. 4. N_{min}-Anhäufung und CO_2-Produktion von Mull „Ottobeuren" (C : N = 20,1; pH = 3,73) ohne (ausgezogene Linien) und mit $CaCO_3$-Zusatz (unterbrochene Linien). Glasinhalt: 27,6 g Tr.S.; Zusatz pro Glas 225 mg $CaCO_3$; pH nach Kalkzusatz bei Versuchsbeginn = 4,85.

* Aus hier nicht beschriebenen Parallelversuchen mit Zusatz von anderen Ca-Salzen, die keine pH-Erhöhung verursachten, ergab sich, dass die $CaCO_3$-Zugabe in den nachfolgend geschilderten Versuchen in erster Linie durch die pH-Erhöhung und nicht durch die Zufuhr von Ca-Ionen auf die mikrobiellen Umsetzungen wirkt.

geschildert. Mit der gewählten Kalkmenge wollten wir einen pH-Wert von etwa 5 erreichen. Wir mischten den kohlensauren Kalk bei Beginn der Betrütung unter das Versuchsmaterial und füllten dann auf den erforderlichen Wassergehalt auf *. Fig. 4 gibt den Verlauf der CO_2-Produktion und der nach NH_4- und NO_3-N getrennt bestimmten N_{min}-Anhäufung wieder. Die pH-Erhöhung hat eine Steigerung der CO_2-Anlieferung zur Folge (oberer Teil von Fig. 4). Sie hält aber nur wenige Wochen an und liegt ebenso wie die späteren, schwankenden Unterschiede zwischen den Versuchsgliedern innerhalb der Fehlergrenze. Die Umsatzaktivität der gekalkten Proben unterscheidet sich also kaum von der des Vergleichshumus. Dies ist nicht erstaunlich, da die Mikroorganismen in Mullhumusproben im allgemeinen bereits ausgezeichnete Lebensbedingungen haben, die sich durch die Kalkung wohl nicht mehr wesentlich bessern.

Die in diesem Versuch teilweise beobachtete, geringe Förderung der Mikroorganismen durch Kalkung ist möglicherweise sogar noch geringer als in der CO_2-Produktion zum Ausdruck kommt, da bei diesem und allen folgenden Kalkungsversuchen die Erhöhung der CO_2-Produktion nicht ausschliesslich durch stärkere Mikrobenaktivität bedingt sein muss. Es kann vielmehr auch ein Teil der als $CaCO_3$ zugegebenen Kohlensäure in diesen sauer reagierenden Proben freigesetzt werden (Schachtschabel [26]). Wir halten diesen Anteil nicht für sehr gross, können ihn aber bei unserer Versuchsanordnung nicht bestimmen.

Die N_{min}-Anhäufung im Mull „Ottobeuren" ist in der gekalkten Serie etwa so hoch wie in den Vergleichsproben. Für die Nitrifikanten schuf die pH-Erhöhung jedoch günstigere Bedingungen: Die im unbehandelten Material nur teilweise Oxydation des angehäuften NH_4-N läuft nun bereits nach wenigen Wochen vollständig ab, so dass im gekalkten Material keine NH_4-N-Anhäufung mehr stattfindet.

Im Gegensatz zu diesem geringen Einfluss der Kalkung auf die N-Mineralisation im Mull hat jedoch die Kalkung von Rohhumus eine tiefgehende Wirkung. Im Rohhumus „Amberg" schnellt, wie aus Fig. 5 (oberer Teil) zu ersehen ist, die CO_2-Produktion nach der Kalkung zunächst rapide in die Höhe. Sie sinkt aber nach etwa 8 Wochen auf das Niveau der Vergleichskurve ab und unterschreitet

* Bei den folgenden Brutversuchen verfuhren wir ebenso.

diese im späteren Verlauf sogar. Die CO_2-Kurve der gekalkten Serie besitzt keinen zweiten Gipfel, während die Vergleichskurve nach etwa 8 Wochen ihre Maximalwerte erreicht. Wie früher geschildert [45], dürfen wir aus diesem Verhalten wahrscheinlich entnehmen, dass die im unbehandelten Rohhumus nach mehreren Wochen Bebrütung aufkommende Pilzaktivität in diesen gekalkten Proben nicht so stark einsetzt. Die in den ersten Wochen erhöhte Mikro-

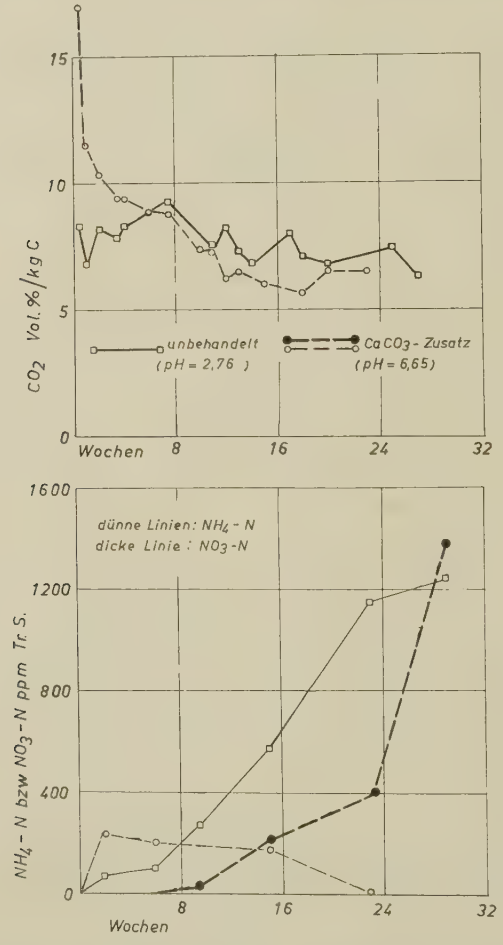

Fig. 5. N_{min}-Anhäufung und CO_2-Produktion von Rohhumus „Amberg" (C : N = 30,5) ohne und mit Kalkzusatz. Glasinhalt: 18,0 g Tr. S.; pH zu Versuchsbeginn ohne Zusatz: 2,76; Zusatz pro Glas: 1,5 g $CaCO_3$ (pH der Probe nach dem Zusatz = 6,65).

organismenaktivität der gekalkten Serie scheint vor allem auf einer Förderung der Bakterien zu beruhen. Sie werden durch höhere pH-Werte wesentlich begünstigt und vermehren sich binnen kürzester Zeit.

Auch die N_{min}-Anhäufung (Fig. 5, unterer Teil) nimmt nach der Kalkung eine deutlich andere Entwicklung. In den ersten zwei Wochen setzt eine starke NH_4-N-Anlieferung ein. Sie übersteigt die der Vergleichsproben um das Doppelte. Die NH_4-N-Mengen sinken dann aber stetig ab, weil nach einigen Wochen Nitrifikation einsetzt. Dies zeigt die bald steil ansteigende NO_3-N-Kurve in Fig. 5. Wichtig erscheint uns hierbei, dass Nitrat erst einige Zeit nach dem Abfall der NH_4-N-Kurve nachzuweisen ist. Möglicherweise tritt inzwischen eine stärkere Immobilisation von NH_4-N durch die nun zur Entwicklung kommenden Nitrifikanten ein.

Addiert man die in Fig. 5 getrennt aufgetragenen NH_4-N- und NO_3-N-Mengen der gekalkten Serie, so erhält man eine Anhäufungskurve, die zeitweilig sogar unter die der Vergleichsserie sinkt (vgl. auch Fig. 7). Wir beobachten also in den gekalkten Proben nach der Anfangserhöhung der N_{min}-Anlieferung eine zwar nur vorübergehende, aber deutliche Depression der N_{min}-Werte. Auch Wheeler u. Yemm [32] beschreiben von ihren Brutversuchen mit Perkolationstechnik derartige Depressionen der N_{min}-Kurven während der Periode der einsetzenden Nitrifikation. Sie deuten dieses zeitweilige N_{min}-Defizit als Immobilisationseffekt, da bereits mit Nitrifikanten angereicherte Brutproben zugesetzten NH_4-N *ohne* Absinken der Werte verarbeiteten. Allerdings zeigen die Gesamt-N_{min}-Kurven bei Wheeler u. Yemm sogar in mit Nitrifikanten angereicherten Proben mitunter Dellen. Daher ist bei ihren und unseren Versuchen die Möglichkeit von gasförmigen N-Verlusten, wie sie Gerretsen u. de Hoop [8] schildern, nicht ausgeschlossen (Entstehung von Stickoxyden durch Reaktion von NO_2 mit NH_4).

Wir untersuchten den Einfluss der pH-Erhöhung durch Kalkung an einer Reihe weiterer Rohhumus- und Moderproben. Ein Versuch mit gestaffelten Kalkgaben zu Rohhumus „München-Süd" sei hiervon noch aufgeführt. Dieser Fichten-Rohhumus (Fig. 6 und 7) hatte nach der Entnahme von Standort bereits einige Monate kühl gelagert und während dieser Vorbebrütung 250 ppm NH_4-N angehäuft. Die Erhöhung der pH-Werte (siehe Figuren-Legende) durch

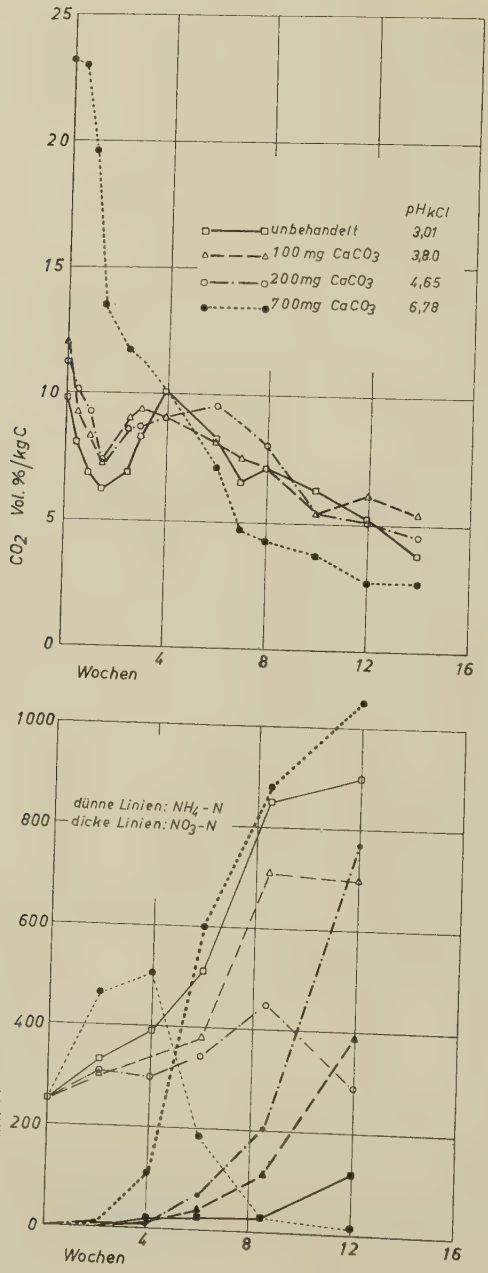

Fig. 6. N_{min}-Anhäufung (NH_4-N und NO_3-N getrennt aufgetragen) und CO_2-Produktion von vorbebrütetem Fichten-Rohhumus „München-Süd" (C : N = = 25,7) bei gestaffelten $CaCO_3$-Gaben.

die Kalkung verursacht eine gesteigerte NO_3-Bildung. Je stärker der pH-Wert gehoben wird, desto stürmischer setzt die NO_3-Anhäufung ein. Die in den ersten Wochen der Bebrütung angelieferten NH_4-N-Mengen nehmen nach Beginn der Nitrifikation umso stärker ab, je mehr NO_3-N auftritt. Sogar im unbehandelten Vergleichshumus beginnt trotz des niedrigen pH-Wertes von 3,01 nach dem Ansetzen zum Brutversuch bei 20° C die Nitratbildung.

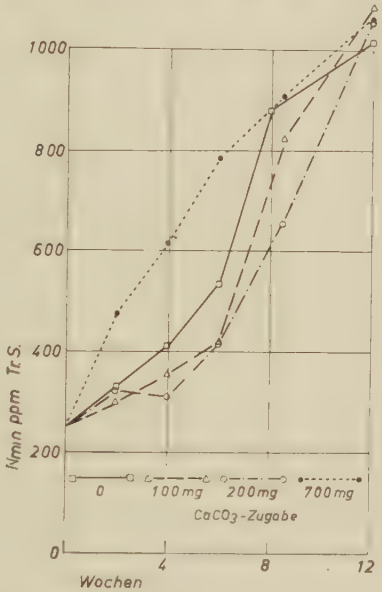

Fig. 7. N_{min}-Anhäufung (Gesamt-Werte) von vorbebrütetem Fichten-Rohhumus „München-Süd" bei gestaffelten Kalkgaben.

Ursache dürfte die lange Vorbebrütung unter relativ günstigen Feuchtigkeits- und Temperaturbedingungen sein, während der offenbar allmählich eine gewisse Vermehrung von Nitrifikanten stattfand. Sie wird durch die höhere Temperatur im Brutversuch dann stärker angeregt. Auch Ehrhardt [7] beobachtete in stark saurem Rohhumus (pH = 2,8) erst nach sehr langer Brutdauer mitunter eine beginnende Nitrifikation.

In Fig. 7 ist die Summe von NH_4- und NO_3-N eigens aufgetragen. Wir sehen, dass nach 12 Wochen Versuchsdauer alle Serien fast gleiche N_{min}-Mengen angehäuft haben. Bei den beiden geringen Kalkgaben ist die oben (Fig. 5) erwähnte N_{min}-Depression während

des Anlaufens der Nitratbildung gut erkennbar *. Die Serie mit der hohen Kalkgabe zeigt diese Erscheinung jedoch nicht. Das entspricht den Beobachtungen von Gerretsen u. de Hoop [8], nach denen gasförmige N-Verluste bei einsetzender Nitrifikation zwar im pH-Bereich von 4 bis 5 möglich sind, jedoch ausbleiben, wenn der pH-Wert über 6 liegt.

Solche N-Verluste in schwach aufgekalkten Proben lassen sich nach Laatsch [21] auch durch Zerfall von gebildetem Nitrit erklären, das nicht zu Nitrat weiter oxydiert wird. Diese Weiteroxydation unterbleibt möglicherweise in unseren Proben zeitweilig, wenn zwar der zur Umwandlung von NH_4 in NO_2 nötige rH-Wert von 0,35–0,40 (bei pH = 7) erreicht wird, das Redoxpotential infolge der Anwesenheit reduzierend wirkender Huminstoffe aber nicht weiter steigen kann. Zur Oxydation von NO_2 zu NO_3 ist nach Mortimer [24] nämlich ein höherer rH-Wert von 0,40 bis 0,45 erforderlich. Wir konnten zwar in unseren Versuchen kein Nitrit nachweisen; dies spricht jedoch nicht gegen die angedeutete Verlustmöglichkeit, da der Zerfall des Nitrits u.U. sofort nach dessen Bildung erfolgen kann.

Betrachten wir nun die CO_2-Kurven in Fig. 6 (oberer Teil). Es fällt auf, dass die beiden schwachen Kalkgaben nur eine geringe Erhöhung der Werte bewirkten. Die stark gekalkte Serie erreicht hingegen zunächst eine sehr grosse CO_2-Produktion, sinkt dann aber durchlaufend unter die Vergleichswerte ab. Im Gegensatz zur unbehandelten Serie und den beiden schwach gekalkten Versuchsgliedern zeigt die Atmungskurve des mit 700 mg $CaCO_3$ versetzten Materials keinen zweiten Gipfel. Wir nehmen auf Grund unserer früheren Ausführungen [45] an, dass dieser abweichende Kurvenverlauf überwiegend durch die rasch und stark einsetzende Bakterientätigkeit bedingt ist. Offenbar ist zu einer solchen mikrobiellen Umstellung eine ziemlich grosse pH-Erhöhung nötig, denn die beiden schwach gekalkten Serien zeigen (ebenso wie das unbehandelte Versuchsglied) noch den 2. Gipfel der CO_2-Kurve, der das Aufleben der Pilztätigkeit andeutet.

Alle durchgeführten Laborbrutversuche mit Kalkzusatz zu Rohhumus und Moder erlauben folgende Auswertung. *Die Anhebung des pH-Werts bewirkt, wie aus der CO_2-Produktion ersichtlich,*

* Vergl. hierzu auch Hiltbold u. Adams [15].

zunächst eine starke Steigerung des Bruttoumsatzes. Sie ist umso grösser, je mehr wir den pH-Wert erhöhen. Wie auch Vandecaveye[30] Duchaufour u. Mangenot[3] und Duchaufour *et al.*[4] nachwiesen, wird sie *hauptsächlich durch die Entwicklung von Bakterien getragen. Erhöht man den pH-Wert stark (bis über 6,0), so verhindert eine stark einsetzende Bakterienaktivität das im sauren Rohhumus sonst nach einigen Wochen Versuchsdauer anzunehmende Aufleben der Pilztätigkeit. Die anfangs sehr gesteigerte Abbauintensität der stark gekalkten Proben sinkt innerhalb etwa 8 Wochen auf oder unter die Werte der Vergleichsserie ab. Eine Zunahme der Gesamt-N_{min}-Anhäufung ist nur bei hohen Kalkgaben und nur in den ersten 1 bis 3 Wochen zu verzeichnen (vgl.* [2]*). Die schwach gekalkten Proben zeigen vor dem vollen Einsetzen der Nitrifikation eine Depression der N_{min}-Kurven. Die Werte liegen in dieser Zeit beträchtlich unter denen der Vergleichsserie. Wahrscheinlich sind gasförmige N-Verluste die Ursache dieses zeitweiligen Defizits. Nach etwa 12 Wochen Versuchsdauer erreichen die gekalkten Proben wieder die N_{min}-Werte des unbehandelten Humus.*

Die N_{min}-Bilanz nach längerer Versuchsdauer ist bei gekalkten und nicht behandelten Proben also ziemlich gleich; die pH-Erhöhung führt lediglich zu einer weitgehenden Umwandlung des sonst als NH_4-N angehäuften Mineralstickstoffs in Nitrat. Solche Ergebnisse überraschen; sie werden jedoch durch andere Untersuchungen bestätigt (z.B. Kaila *et. al.*[17], Kaila u. Soini[18]). Bei diesen Resultaten ist jedoch zu bedenken, dass sie zunächst nur für die Bedingungen des Laborbrutversuchs gelten, wo eine Beteiligung der Bodenfauna weitgehend ausgeschaltet ist.

Führt man eine Kalkung am Standort durch, so laufen zwar wohl grundsätzlich die gleichen mikrobiellen Vorgänge ab; hinzu kommt aber die Mitwirkung der Bodentiere. Diese spielen bei der Verarbeitung des organischen Materials eine grosse Rolle (siehe Wittich[34,37]). Auf Änderungen des Milieus kann die Bodenfauna armer Waldstandorte, wie z.B. Ronde[25] zeigte, mit entscheidenden Umstellungen reagieren. So ist anzunehmen, dass auf ein starkes Aufleben der Mikroorganismentätigkeit nach einer Kalkung am Standort rasch auch eine erhöhte Tiertätigkeit folgt. Sogar bislang fehlende Artengruppen können sich dabei erstaunlich schnell einfinden[25]. Durch die Tätigkeit der Bodenfauna wird am Standort dann mehr organische Substanz aufbereitet und für die

Mikroorganismen verfügbar (Ulrich [29], Dunger [6]). Ausserdem bewirken die Bodentiere eine Vermischung des Bodenmaterials. Diese Vorgänge können die Nettomineralisation des organisch gebundenen Stickstoffs nach einer Kalkung am Standort daher (im Gegensatz zum Laborbrutversuch) entsprechend verstärken.

Es ist aber auch das Gegenteil, nämlich eine N-Sperre, denkbar. Zweifellos wird die Bruttomineralisation infolge erhöhter Tier- und Mikroorganismentätigkeit in jedem Fall steigen. Wieviel N_{min} dabei als Nettobetrag angeliefert wird, hängt jedoch vom Ausmass der N-Immobilisation ab. Diese N-Festlegung könnte gerade auch durch die zunehmende Tiermasse stark erhöht werden. Wird durch die Tiertätigkeit noch dazu viel zellulosehaltige Substanz für die Mikroorganismen verwertbar, so steigt die N-Immobilisation entsprechend weiter an (vergl. [44]). Wir dürfen wohl vor allem dann, wenn hohe Kalkgaben eine starke und plötzliche Milieuänderung hervorrufen, mit dem Auftreten einer N-Sperre rechnen. Denn in solchen Fällen wird sich die Tier- und Mikroorganismenmasse rasch unter gleichzeitig hoher N-Festlegung vermehren. Erst wenn später die Biomasse ungefähr konstant bleibt, ist eine Zunahme der Nettomineralisation zu erwarten. Diese N_{min}-Mehranlieferung kann dann jedoch lange Zeit andauern. Ausserdem ist beim Schluss von den vorliegenden Ergebnissen der Laborbrutversuche auf die am Standort ablaufenden Vorgänge zu bedenken, dass dort auch die beim Einsetzen der Nitrifikation beobachtete N_{min}-Depression u.U. eine Rolle spielt und sich in einer vorübergehend geringeren Stickstoff-Nachlieferung auswirkt.

Kehren wir zurück zu den besprochenen Laborversuchen. Hier trat auch bei Aufkalkung bis nahe an den Neutralpunkt keine N-Sperre ein. Der früher ([44], Fig. 2) angeführte Versuch mit dem am Standort gekalkten Rohhumus „Schwabach" zeigte jedoch dieses Phänomen. Auch Wittich [36] und Duchaufour u. Guinaudeau [5] schildern eine solche Möglichkeit. Manche Fehlschläge von Meliorations- und Düngungsmaßnahmen im Walde lassen ebenfalls eine N-Sperre infolge vollständiger und lange anhaltender Immobilisation des mineralisierten Stickstoffs nach übermässiger Kalkung vermuten.

ZUSAMMENFASSUNG

In Laborbrutversuchen mit Waldhumusmaterial wird die Beziehung zwischen pH-Wert und Mineralstickstoff-Nachlieferung geprüft. Rohhumus- und Moderproben lassen eine schwache positive Korrelation zwischen pH-Wert und Mineralstickstoff-Anhäufung erkennen, für Mullproben ist ein negativer Zusammenhang angedeutet. Zwischen pH-Wert und C : N-Verhältnis besteht eine enge Beziehung. Je niedriger der pH-Wert ist, umso mehr erweitert sich das C : N-Verhältnis.

Während der Bebrütung steigt der pH-Wert bei Anhäufung von NH_4-N und fällt bei Anhäufung von NO_3-N.

Die Kalkung von Mullhumus verändert die Brutto- und Netto-beträge der N-Mineralisation kaum. In stark sauren Rohhumus- und Moderproben bewirkt die pH-Erhöhung durch Kalkung in den ersten Wochen der Bebrütung eine beträchtliche Steigerung des Bruttoumsatzes. Die N_{min}-Anhäufung ist nur bei starker pH-Erhöhung (auf über 6) anfangs gefördert. Werden durch die Kalkung pH-Werte von 4 bis 5 erreicht, so treten vorübergehende Depressionen der N_{min}-Anhäufungskurve ein, die auf N-Verluste deuten. Nach längerer Versuchsdauer ist die Gesamt-N_{min}-Anhäufung in gekalkten und unbehandelten Proben annähernd gleich. Die Nitrifikation des angehäuften NH_4-N setzt umso rascher ein, je stärker der pH-Wert erhöht wurde.

SUMMARY

The relation between pH value and nitrogen mineralization was investigated in laboratory incubation studies with humus material of forests in Bavaria. Samples of raw humus and moss humus show a weak positive correlation between pH value and mineral nitrogen accumulation. For mull humus samples a negative relation is indicated. There exists a close correlation between pH value and C : N ratio: the lower the pH value, the more the C : N ratio is expanded.

During incubation the pH value increases when NH_4-N is accumulated, and decreases when NO_3-N is accumulated.

Liming of mull humus hardly alters the gross- and net-mineralization.

In very acid raw humus and moss humus, raising the pH by liming, in the first weeks of incubation causes a considerable increase of the gross-turnover. The mineral nitrogen accumulation is only increased initially by a marked rise of pH (to over 6). If pH values of 4 to 5 are reached by liming, transitory depressions occur in the mineral nitrogen accumulation curve, which indicate nitrogen losses. After a long incubation period the sum of accumulated NH_4-N and NO_3-N in the limed series is about the same as in the untreated samples. Nitrification of accumulated NH_4-N proceeds more quickly as the pH value is raised.

Verf. dankt Herrn Professor Dr. W. Laatsch für wertvolle Diskussionen, Fräulein I. Schild und Fräulein K. Zellner für die Durchführung der Laborarbeiten.

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IN MEMORIAM
PROF. DR. MARGARETHA G. MES

Prof Dr. Margaretha G. Mes died on July 25, 1959, after a long illness borne with great fortitude. She was a Consulting Editor of "Plant and Soil".

Margaretha Gerarda Mes was born in Dubbeldam, Holland, in 1905. Her family emigrated to the U.S.A. in 1912 and spent two years there, after which they came to South Africa. She received her undergraduate training at the Transvaal University College under Prof. Bremekamp, and she was awarded a B.Sc. (*cum laude*) in 1924. She continued her studies under Prof. Johanna Westerdyk at the University of Utrecht, where she was awarded a Ph.D. (*cum laude*) in 1930. Her doctoral dissertation, entitled "Fisiologiese Siektesimptome in Tabak", was subsequently reprinted in the Phytopathologische Zeitschrift. This choice of subject for her doctoral dissertation was symbolic, for throughout her lifetime she never lost sight of the relationship of plant physiology and biochemistry to agriculture.

In 1931 she was granted a Junior Fellowship by the International Federation of University Women in order to spend a year at the University of California, Berkeley, under Prof. Hoagland. After this, she travelled in Japan and China, where she planned to teach; but the offer of a lectureship in the Department of Botany at the University of Pretoria prompted her return to South Africa in 1939. She became head of the Department of Botany in 1944, when she was only 39 years of age. She was the first woman to be appointed head of a department in a faculty of Science at a South African University.

Besides fulfilling her duties as a lecturer, Prof. Mes found time to work untiringly for the establishment of a research laboratory

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for plant physiological studies at the University of Pretoria. In spite of scepticism and even opposition, she began collecting money. Large donations from the Rockefeller Foundation, the Bertha Stoneman Estate, her father and various friends, and smaller donations from students, schools, businessmen and other individuals, were supplemented by an equal sum of money from the South African Government. With this money she was able to realize her dream of building a Plant Physiological Research Institute at the University of Pretoria, the only institute of its kind in Africa. The facilities include a phytotron, which was built in 1948–1949; at that time the only other phytotron in the world was Prof. Went's in California. In her honour the Institute has been named the "Margaretha Mes Institute for Plant Physiology and Biochemistry".

Prof. Mes worked as a research fellow in laboratories in the U.S.A. on three occassions between 1941 and 1955. Her last visit was in 1954–1955, when she was awarded a research fellowship at the California Institute of Technology by the I.B.E.C. Research Institute in order to study certain problems in the flowering of coffee. In a series of elegant experiments, she demonstrated that dormancy in coffee flower buds is caused by an inadequate supply of water to the buds, and that they begin to grow immediately after water is supplied to them directly, either by rain or by overhead irrigation. An account of this investigation was published in Portugaliae Acta Biologica in 1957 and was subsequently translated into Spanish by the I.B.E.C. Institute.

Prof. Mes was a member of the Board of Trustees of the National Veld Trust and of the Chemical Research Advisory Committee of the Council for Scientific and Industrial Research. In 1941 she was elected President of the South African Biological Society and in 1941 1942 she was President of Section C of The South African Association for the Advancement of Science. In 1956, she was awarded the Senior Captain Scott Medal by the South African Biological Society. Shortly before her death she was invited to take part in the Symposium on "Plant Growth under Controlled Environmental Conditions" at the 1959 International Botanical Congress.

An examination of her publications indicates the numerous fields in which Prof. Mes conducted research. Her major interests were

water relations, germination, nitrogen fixation in legumes, and the influence of climate on plant growth. Her research was aided by numerous grants from organizations such as the Nuffield Foundation, the Rockefeller Foundation, the Council for Scientific and Industrial Research, and the South African Department of Agriculture.

Apart from her academic activities, Prof. Mes had a wide range of interests. She was particularly fond of the open air and of animal life, and was a keen amateur photographer. A camping holiday with Prof. Mes was an unforgettable experience; her companions gratefully relegated the rôle of *chef* to her and settled back to enjoy *haute cuisine* even in the wilds of Africa.

During the war, Prof. Mes and Miss Dien Wijers, a lecturer at the University of Pretoria, established a Red Cross Correspondence Service in order to provide contact, after the invasion of Holland, between Hollanders in South Africa and overseas.

For many years Prof. Mes had known that she was suffering from an incurable disease; yet this did not affect her sense of humour or her infinite patience with her colleagues and students. This knowledge that she had only a limited time to accomplish what she felt was necessary was perhaps partly responsible for her tremendous drive and energy. In the three weeks before her death, she completed three articles in spite of the fact that she was bedridden.

Several generations of students remember Prof. Mes with affection and gratitude. In her lectures she stressed the importance of detachment, curiosity, imagination and an analytical mind in a scientist, and did not allow principles to be bogged down with details. The source of inspiration that she remains to those who came into contact with her, and the Institute that bears her name, serve as monuments to the memory of Prof. Mes.

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A METHOD OF DIAGNOSING THE SULPHUR NUTRITION STATUS OF HERBAGE

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INTRODUCTORY

In herbage production the amount of sulphur required for the growth and normal functioning of the synthetic activities of the grass plant is considerably lower than the nitrogen demand and sulphur shortage has not been reported as frequently as nitrogen shortage.

Sulphate is absorbed by herbage with more difficulty than other anions and it has been shown that the uptake proceeds even slower in the presence of more mobile anions such as chloride and nitrate [4 5 7].

Therefore it may sometimes be useful to demonstrate that the sulphur supply has been adequate *e.g.* when the herbage has been grown at high rates of nitrogen and chloride application and the results indicate the interference of some other nutritional factor in growth.

In the present communication it is shown that information concerning the sulphur and nitrogen content of the herbage can indicate the adequacy of the sulphur supply. The test has been inferred from compositional data on perennial ryegrass grown at varying nitrate and sulphate supply.

Usually sulphur and nitrogen are absorbed in the form of sulphate

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and nitrate from the soil and subsequently undergo metabolic conversion into organic sulphur and organic nitrogen. Any quantity above that needed to supply sulphate and nitrate to the metabolic process is stored up provisionally as inorganic sulphate and nitrate in the plant.

The organic sulphur and the organic nitrogen formed are built up into the protein substance. This process, if carried to completion, results in the ratio of organic-S to organic-N in the total mass of herbage: $(S/N)_o$ being equal to the ratio of these elements in the protein substance: $(S/N)_p$.

The protein substance contains a variety of proteins, each of them representing a chemical species of definite elementary composition. It is natural to assume that at normal growth the mutual proportion between the individual protein species is constant. In this case the ratio $(S/N)_p$ in the protein substance is constant as well and independent of the concentration of total-S and total-N in the plant material as conditioned by total uptake and plant weight.

On the average, vegetable proteins have been found to contain 1 per cent S and 17 per cent N. These percentage values, after dividing by the atomic weights, yield an atomic ratio of sulphur to nitrogen: $(S/N)_p = 0.025$.

From the preceding considerations it would follow that the atomic ratio of organic-S to organic-N in the total mass of herbage: $(S/N)_o$ equals $(S/N)_p = 0.025$, the organic form of these elements being located in the protein substance. Any shortage in sulphur relative to nitrogen would inhibit protein formation, otherwise the S/N ratio would fall below the value $(S/N)_p$ specific to the protein substance. Also any sulphate absorbed in excess to this proportion would remain unutilised.

A slight complexity must now be considered. The amount of sulphate and nitrate transferred to the organic state is regulated by the rate of metabolism which is a function of growth. It appears, however, that the transformation of the organic form of these elements into proteins is not carried to completion. The remainder, although forming but a minor proportion of the material, is stored up as low-molecular sulphur–nitrogen compounds. In grass this non-protein substance mainly represents sulphuretted amino acids and polypeptides.

On account of the ability of grass to accumulate variable amounts

of non-protein N-metabolites free of sulphur (such as glutamic and aspartic acid) the ratio of S to N in the non-protein organic substance is usually different from the protein ratio $(S/N)_p$ and is subject to variation according to the nutritional status of the plant. Therefore the ratio of organic sulphur to organic nitrogen in the total mass of herbage: $(S/N)_o$ is also different from $(S/N)_p$ and is some function of the nutritional status and the composition of the plant.

The extent to which the ratio $(S/N)_o$ will vary depends on what proportion of organic-S and organic-N is converted into protein substance of constant S/N and on the possible limits of variation of the S/N ratio in the organic non-protein substance of varying S/N. It is known that with normal growth a large proportion of the organic-S and the organic-N is utilised in protein formation. Therefore any change of S/N in the non-protein organic substance cannot be very effective in altering $(S/N)_o$ relative to $(S/N)_p$. Only at more severe S-starvation when protein formation becomes strongly inhibited the ratio $(S/N)_o$ may fall significantly below $(S/N)_p$ as a result of the increased accumulation of non-protein substance lower in sulphur relative to nitrogen.

EVIDENCE ON A SULPHUR–NITROGEN RELATIONSHIP

The above considerations receive confirmation from the data of Ergle and Eaton [10] which have been selected to demonstrate experimental evidence because their experiments have many features in common with the present investigations. Ergle and Eaton have studied the sulphur and nitrogen fractions in cotton grown at varying sulphate supply and constant supply of nitrogen.

To demonstrate the relative alterations resulting from the increase of sulphate supply the data have been plotted as logarithmic ordinates in Fig. 1. Protein-N (N_p) has been chosen as the reference by plotting the values against log N_p as abscissae. Consequently, protein-N yields a straight line which furnishes a convenient reference to the relative alterations of the other constituents. Any constituent which varies proportional to protein-N yields another straight line parallel to protein-N. Its position on the ordinates is determined by the ratio of this constituent to protein-N.

If the ratio of sulphur to nitrogen in the protein substance: $(S/N)_p$ is given by the value 0.025 the values of protein-S (S_p)

should coincide with the dashed line drawn parallel to protein-N (N_p) with an ordinate intercept of 0.025 at $N_p = 1$.

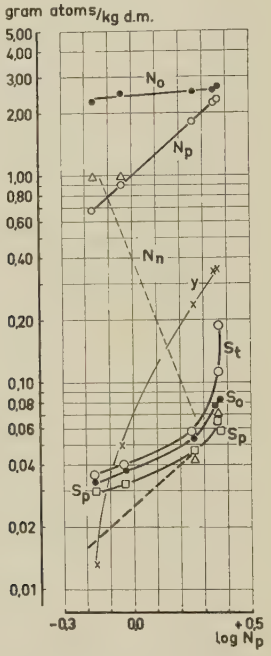

Fig. 1. Sulphur and nitrogen fractions in the leaves of cotton grown at increasing supplies of sulphate, expressed as gram atoms per kilogram dry weight. Calculated from data of Ergle and Eaton [10] on leaves of 65-days-old plants (tables IV and V of the reference). N_o = organic-N; N_p = protein-N; N_n = nitrate-N; S_t = total-S; S_o = organic-S; S_p = protein-S. y = fresh weight per plant for ordinates in kilograms.

The data on protein-sulphur percentage have been recalculated into gram atoms S per kg dry weight of leaves. As expected, values are obtained which coincide quite closely with the dashed line but these are located in the range of higher sulphur levels. At low sulphur the protein-S values are found to be located above the heavy dashed line relating to the proportionality between S and N in the protein substance. This shows that the ratio of S to N in the protein substance is increased at low sulphur relative to the normal value of $(S/N)_p$ found at higher internal sulphur levels.

To demonstrate the advance of S-starvation at the decline of sulphur the fresh weights per plant have been superimposed as curve

y in Fig. 1. It is seen that the lower S-levels relate to severe starvation since plant weight declines considerably. The last two observations at high S refer to adequate S because there is no further increase in plant weight.

Ergle and Eaton have noticed the higher S-content relative to N in the protein substance of the severely S-starved plants. In this stage of S-starvation the make-up of the protein substance has apparently been changed in favour of the protein species of higher S relative to N.

With the increase of sulphur, protein-S (S_p) rises proportional to organic-S (S_o) as shown by the curves which run nearly parallel. This graphically expresses constancy of the ratio of protein-S to organic-S. The levels indicate that about 85 per cent of the organic-S is utilised in the formation of the proteins and that this level of conversion of organic-S into proteins is not significantly altered when S increases from the extremely deficient level up to that of adequate internal supply.

As to the behaviour of total sulphur (S_t) it should be noticed that this quantity nearly equals organic-S in the deficient range, indicating the absence of inorganic sulphate. It is only when sulphur has been increased to the adequate level that the difference ($S_t - S_o$) indicates the presence of 0.05 to 0.1 moles of sulphate per kg dry weight of leaves. At the adequate supply sulphate has been absorbed in excess of the sulphate-assimilation capacity of the plant material. This shows that the conversion of absorbed sulphate into the organic state proceeds to completion only when the uptake remains below the requirements.

The evidence indicates that an adequate sulphate supply is associated with the presence of a finite amount of internal sulphate which tends to nil at the onset of sulphur depletion. Therefore a check on adequate S is furnished by a positive test on the presence of inorganic sulphate in the plant material.

Mention has already been made of the fact that S-deficiency lowers protein formation relative to the production of organic nitrogen. This is graphically expressed as the considerable divergence of the lines representing organic-N (N_o) and protein-N (N_p) at the decrease of sulphur. According to the levels protein-N constitutes about 85 per cent of the organic-N at adequate sulphur. This conversion of organic-N into protein-N continuously drops to

30 per cent at the lowest sulphur level. The reduction of protein formation apparently results from shortage in sulphuretted amino acids, indispensible for the complete constitution of the proteins.

The inhibition of protein formation leads to a marked increase in non-protein organic nitrogen as indicated by the increase of $(N_o - N_p)$ when sulphur declines. Since the non-protein organic substance is lower in S relative to N as compared to protein composition, the ratio of organic sulphur (S_o) to organic nitrogen (N_o) shows a marked decline with the decrease of sulphur. This is apparent from the increase of the linear distance between S_o and N_o towards lower S_o. From the levels shown on the graph it can be inferred that, when S-supplies are adequate $(S/N)_o$ equals 0.029, but that this value drops to 0.014 at the lowest S level *.

From the foregoing evidence certain conclusions can be drawn. In the first place it is obvious that although the ratio of S to N in the protein substance $(S/N)_p$ is subject to alteration at more severe S-starvation, this ratio comes quite close to the expected value of 0.025 when S-supplies are adequate or under conditions of moderate S-deficiency. Secondly at adequate sulphur levels the ratio of organic-S to organic-N is only slightly higher than 0.025 (reading the graph yields $(S/N)_o = 0.029$ at the highest S-level) and at a moderate deficiency level slightly lower than 0.025 (reading the graph yields $(S/N)_o = 0.023$ at $\log N_p = 0.25$). With the further advance of sulphur shortage up to severe starvation there is a marked decline of $(S/N)_o$ relative to $(S/N)_p$ [reading the graph yields $(S/N)_o = 0.014$ at $\log N_p = -0.16$]. Briefly, at an adequate supply of sulphate by uptake $(S/N)_o \geqslant (S/N)_p$ while at sulphur shortage relative to nitrogen utilisation and growth $(S/N)_o < (S/N)_p$.

Finally, in the range of a more adequate supply including moderate S-deficiency, any increase of protein-N gives rise to a proportional increase of protein-S. Since the ratio of organic-S to protein-S remains unchanged, it follows that organic-S also increases proportionally to protein-N. This is expressed graphically as the parallel trend of N_p, S_p and S_o in the range beyond $\log N_p = 0.25$. From this it follows that the sulphur requirements depend mainly on the utilisation of nitrogen in protein formation.

* Ratio values can be conveniently measured by marking the levels to be compared on a separate strip, and in the case of ratio values < 1, by shifting the upper mark on the ordinate level 1.00 and reading the ordinate at the lower mark.

Another matter may conveniently be mentioned at this point. The graph shows that although S-starvation slightly lowers organic-N (N_o) this effect on the amount of organic nitrogen formed per unit weight of plant material signifies little as to the relative alterations of the other constituents. However, nitrate nitrogen (N_n) increases considerably with reduced sulphur supply. This might suggest an accumulation of nitrate due to some specific interference of S-starvation in the process of nitrate reduction, which is not reflected in a comparable decline of organic-N because its supply by translocation of N-metabolites from other parts of the plant is increased. But nitrate is hardly a final test in a case such as this and should not be taken as true evidence on any specific role of sulphur in the process of nitrate reduction.

A corollary to this result is that with equal, initial nitrate supplies each treatment which reduces plant weight results in a slower decrease of the external nitrate level due to a reduced uptake.

At that stage of the experiment a reduced plant weight is associated with a relatively higher internal nitrate level [6], since the internal nitrate levels actually reflect the gradient in available nitrate brought about through differences in uptake associated with different plant weights. Sometimes, the effect throws doubt on the evidence derived from nitrate in deficient plants as to a specific role of the element concerned in the biochemical pathway of nitrate reduction, as done *e.g.* recently by Amin and Joham [1].

EXPERIMENTAL

Materials and methods

For each experiment a series of six nutrient solutions was prepared in which the nitrate and sulphate supply was varied inversely by adding x me KNO_3 + $(a - x)$ me K_2SO_4 per each 6 liters solution. In Experiment A the amount a was 25 and x was varied from 0 to 25, the first treatment including a supply of 0 nitrate and 25 me sulphate, the last treatment 25 me nitrate and 0 sulphate. In the Experiments B to G x was varied from 0 to 50, and a was 50. There was given, in addition, a constant extra supply of 20 me nitrate. Thus in the experiments B to G the nitrate supply increased from 20 to 70 me and the sulphate supply decreased from 50 to 0 me per 6 liter solution.

K, Mg, Ca, and P were added in constant amounts to each of the solutions.

Glass containers of 6 liter capacity were used and provided with perforated plastic covers (50 perforations of 1 cm diameter). Plants of perennial ryegrass

(*Lolium perenne*), previously grown in the garden, were made up into bundles of suitable size and the soil adhering to the roots was removed by thorough rinsing in running tap water. The bundles were mounted in the holes of the covers, the plants clipped off at a height of 3 cm above the cover, corresponding to a plant length of 6 cm from the root base, and the clipped plants placed on the experimental solutions. During the subsequent regrowth period of three weeks the volume of the solutions was daily made up to 6 liters by adding demineralised water. Thereafter the herbage produced was collected by clipping at the initial height of 3 cm above the covers. Recently Said [12] has described this procedure more in detail. The experiments were carried out under greenhouse conditions from July till October.

The herbage was oven dried (105°C), ground, and stored in glass bottles in the dark untill required for chemical examination.

Total nitrogen was determined as ammonia after Kjeldahl digestion, using Cu and Se as catalysts. The procedure is known to include a variable fraction of the nitrate-N in the sample, from 40 to 80 per cent being recovered as ammonia [8].

Protein-nitrogen was found by digesting a 1-gram sample in 60 ml water during 1 hour on the waterbath, cooling, adding 25 ml trichloracetic acid (TCA) 25%, making up to 100 ml and leaving overnight. The residue, after washing with 1% TCA, was analysed for nitrogen using the Kjeldahl method.

For protein sulphur the TCA-insoluble residue was wet ashed with nitric–perchloric acid and, after dissolving the residue the sulphate was precipitated with Ba. Barium sulphate was either weighed after ignition in a platinum crucible (gravimetrically) or calculated from the reduction in optical density of added standard chromate brought about through subsequent Ba-chromate precipitation (colorimetrically).

Total sulphur was determined after wet-ashing the sample with nitric-perchloric acid, precipitation with standard Ba added in excess, followed by adding standard chromate equivalent to total Ba and measuring the excess chromate by means of the optical density at 400 mμ. This was done according to the instructions of Deys and Bosman (unpublished), resulting in a linear relation between optical density and sulphur content of sample.

Inorganic sulphate was determined according to Bergh [2] by treating a 2-g sample with 100 ml 20% HCl, evaporating to dryness on a waterbath, making up with water to 200 ml, filtering and precipitating the sulphate with excess Ba in an aliquot volume of the filtrate. After ignition in a platinum crucible the Ba-sulphate was weighed.

For nitrate nitrogen the xylenol procedure in the modification of Dijkshoorn and Lampe [9] was used.

Organic sulphur was obtained by difference between total sulphur and inorganic sulphur.

The results have been expressed as gram atoms S or N per kilogram dry material. The use of weight-percentage values, although more often used, might have led to confusion on account of the different chemical species involved.

Results and discussion

The present work seeks to provide a means for evaluating the sulphur-nutrition status of grass from information concerning the quantities of sulphur and nitrogen in the herbage. Attention will be confined largely to the ratio of organic-S to organic-N which is mainly determined by the elementary composition of the protein substance. The amount of organic-S present at adequate sulphur supply and at a given nitrogen status is considered to represent the sulphur requirement at this particular level of nitrogen utilisation in growth.

As mentioned before the ratio of S to N in the protein substance largely regulates the proportion in which the organic form of these elements occurs. From the present results an average value is obtained by plotting protein-S against protein-N (Fig. 2). On the assumption that these quantities vary proportional to each other the result has been indicated by a straight line through the origin. The slope of this line indicates that on the average the atomic ratio of sulphur to nitrogen in the protein substance: $(S/N)_p = 0.027$.

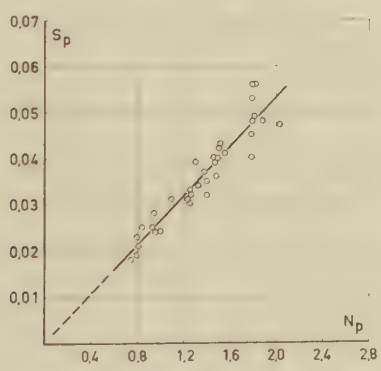

Fig. 2. Gram atoms protein-S plotted against gram atoms protein-N per kilogram oven-dry herbage of perennial ryegrass produced at varying nitrate and sulphate supplies in the seven experiments on nutrient solutions.

The other constituents are shown graphically in Fig. 3 for each experiment separately. They have been plotted in a manner similar to that used in the discussion of the results of Ergle and Eaton.

Here, the log total-N values have been plotted as abscissae against the sulphur and nitrogen fractions as logarithmic ordinates. Accordingly total-N (N_t) yields a straight line of constant slope,

which, in itself, furnishes a convenient uniformity in the results of the different experiments. The difference in the gradients of total-N relative to the amount of nitrate supplied in the solutions is only evident from the difference in range of abscissae occupied by each of the Experiments A, B, C *etc.*

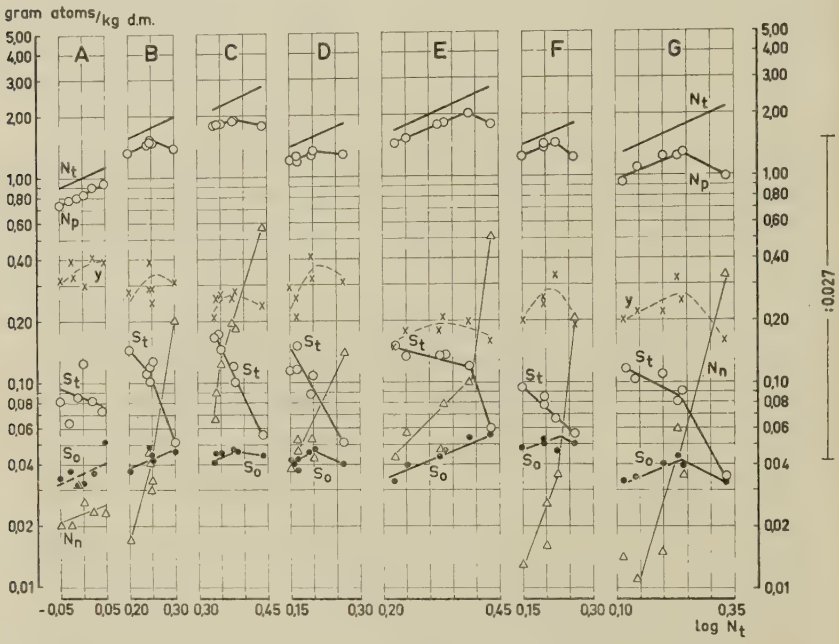

Fig. 3. The sulphur and nitrogen fractions in perennial ryegrass grown at increasing nitrate and decreasing sulphate supplies. The data have been plotted as logarithmic ordinates against log total-N as abscissae and resulted from 7 different experiments A, B, C etc. N_t = total-N; N_p = protein-N; N_n = nitrate-N; S_t = total-S; S_o = organic-S, expressed as gram atoms per kilogram oven-dry herbage. The length of the line on the right-hand side of the graph indicates the ratio 0.027. Y = yield, g $\times$ 10^2 of dry matter per pot.

The main response to the treatments is the increase in N which is associated with a decline in total sulphur. It is seen that all the treatments have yielded amounts of total sulphur (S_t) larger than organic sulphur (S_o). This excess, representing inorganic sulphate, eventually becomes very small – or negligible compared with the analytical error – in the minus-S treatments of the experiments

B to G, where total-S has been lowered sufficiently by the regrowth without sulphate supply. These plants were slightly chlorotic - a visual symptom of sulphur shortage.

Chlorosis was not observed in the minus-S treatment of Experiment A. In this case a sufficient supply resulted from the translocation of sulphate reserves in the clipped plants to the growing herbage. For this reason the nitrate supply was increased in the later Experiments B to G with the object of raising the S-requirements by the increase of growth and nitrogen utilisation. Adequate internal sulphate supply in the minus-S treatment of Experiment A is confirmed by the analytical evidence. Inorganic sulphate is found to be present in an amount, corresponding to 0.023 gram atoms S per kg dry weight, which clearly indicates an adequate supply.

The response of organic sulphur (S_o) to the increase of nitrogen has been represented by lines drawn parallel to protein nitrogen (N_p). This interpretation of the data implies that organic-S rises proportionally to protein-N. Further, where sulphur supplies are adequate, total-N (N_t) varies proportionally to protein-N. These conditions have been graphically expressed as the parallelism between S_o, N_p, and N_t.

This relationship holds good for adequate supplies of sulphur. It does not apply in the minus-S treatments of the Experiments B to G. Here, S-deficiency has interfered with protein formation as is apparent from the fall of protein-N from its initially higher level in the preceding treatment of still adequate sulphur supply.

The important deduction to be drawn from these observations is that the sulphur requirements increase proportional to the nitrogen level. When nitrogen is increased and sulphur is diminished the resulting shift in the sulphate balance of uptake and utilisation eventually leads to nearly complete consumption of the sulphate absorbed. In this case further growth leads to S-deficiency and protein formation is inhibited relative to nitrogen utilisation.

The numerical evaluation of the ratio of S to N in the protein substance resulted in $(S/N)_p = 0{,}027$. It was inferred from the data of Ergle and Eaton that at adequate S or at moderate deficiency the ratio $(S/N)_p$ nearly equals the ratio of organic-S to organic-N: $(S/N)_o$.

Generally, nitrate in the herbage is low as compared to Kjeldahl total-N and is only partly recovered as ammonia in the Kjeldahl

procedure. Therefore the ratio of organic-S to total-N becomes sufficiently identifiable with $(S/N)_o$ at lower internal nitrate levels. Because there is no data available on organic-N * this approximation is used in the further evaluation of the sulphur–nitrogen relationship in the utilisation of these elements.

The constancy of the ratio of organic-S to total-N has been indicated by the parallelism of the corresponding lines in the range of adequate sulphur supply. Its numerical value can be measured from the graphs. The values arrived at in this way are, in the order A, B, C *etc.*:— 0.037; 0.025; 0.020; 0.029; 0.021; 0.033 and 0.025. The average of these values is 0.027. These results indicate that sulphur and nitrogen are assimilated in a proportion similar to the ratio of S to N in protein formation.

There remains for consideration the increase of nitrate-N with the increase of total nitrogen. This effect merely reflects the increase in nitrate supply inherent to the treatments and is not connected with the variation in sulphur supply. This view is sufficiently supported by the fact that plotting the nitrate data against total-N yields a curve similar to that obtained by Said [12] for herbage grown at increasing rates of nitrate fertilisation.

Evaluation of the sulphur-nutrition status from total sulphur and Kjeldahl nitrogen in herbage

By coupling together the results of Ergle and Eaton [10] on cotton and those obtained in the present investigations on perennial ryegrass the evidence is sufficient to prove that sulphur and nitrogen are utilised in growth according to a quite definite stœchiometric relation which closely agrees with the elementary composition of the principal consumer of these elements – the protein substance. The ability of herbage to utilise its internal inorganic sulphate nearly completely after the exhaustion of the external source implies that true sulphur deficiency is not perceptible in herbage until after the total sulphur equals the organic sulphur.

This evidence presents features of practical interest to nutritional diagnosis. If organic-N is known, *organic*-S is given by 0.027 ×

* Under this heading are grouped the nitrogen compounds minus nitrate and nitrite. It refers to the moles of nitrate reduced to the reduction stage of NH_3, per kilogram dry-weight production and, accordingly, includes any ammonium ions present in the herbage.

× *organic*-N *. This is the internal sulphur level actually required for the complete constitution of the plant material. If sulphur is present in excess of this basic requirement, the sulphate supply is adequate. Lower total-sulphur values indicate S-deficiency.

Information on nitrogen in herbage generally refers to figures obtained by the Kjeldahl method. Since nitrate is mostly low as compared to total-N, the Kjeldahl values are sufficiently accurate estimates of organic-N. A rough estimate of the sulphur requirements may obviously be obtained by multiplying the Kjeldahl-N content by 0.027.

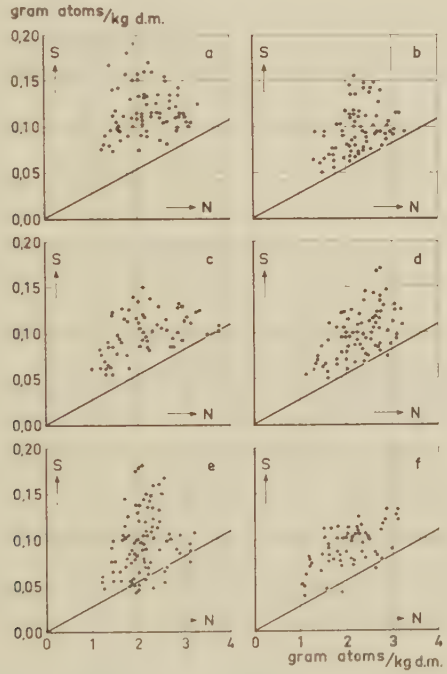

Fig. 4. Data on total-S (ordinates) and Kjeldahl-N (abscissae) gram atoms per kg dry weight of field-grown herbage from pastures of various locations. Calculated from data of Brandsma [3] (b), Posthuma [11] (e and f), Van Burg [13] (a and c) and from the experimental farms "Stikstofproefbedrijven" [14] (d). The mineral S-requirements are indicated by the line S = 0.027 × N.

* The proportionality factor represents the average value found for well-nourished grass. It is necessary to emphasise the simplification which is introduced here, because actually the factor was found to vary between 0.037 and 0.020. This is a between-experiment variability which may have its origin in varying physiological conditions of the plants submitted to similar treatments.

If put into the form of a graph where the ordinates represent gram atoms S and the abscissae gram atoms Kjeldahl-N, observations relating to adequate sulphate supply will become located above the line S = 0.027 × N. Values located below this transition line throw doubt on whether the internal sulphate supply by uptake has been adequate relative to nitrogen utilisation and growth.

An arbitrary set of data on field-grown herbage from pastures of various locations in the Netherlands have been plotted in this way in Fig. 4. These data do not pretend in any way to represent typical or average conditions, but they show that usually sulphate has been absorbed in amounts beyond the minimal requirements indicated by the transition line. Those few points located below the line might have deserved attention with respect to adequacy of sulphur supply. This could have been easily checked upon by carrying out a test on the absence of inorganic sulphate in the herbage samples.

SUMMARY

The present investigations were undertaken to estimate rates of utilisation of absorbed sulphate at varying internal nitrogen levels in growing herbage.

Previously clipped plants of perennial ryegrass were maintained on solutions with increasing nitrate and equivalently decreasing sulphate supply and analysis made of the three-weeks-old herbage for total, organic, and protein nitrogen and sulphur.

It was found that S and N are utilised in the building up of protein substance in an atomic ratio $(S/N)_p = 0.027$. Since the major proportion of the organic form of these elements is converted into proteins the ratio of organic-S to organic-N in the total mass of herbage: $(S/N)_o$ approximately equals $(S/N)_p = 0.027$. Any sulphate absorbed in excess of this proportion is stored up as inorganic sulphate.

From these results the minimal sulphur requirements for normal growth would show an organic sulphur content which is given by 0.027 × organic-N, expressed as gram atoms of the elements. If nitrate is low relative to total-N, Kjeldahl-N becomes sufficiently identifiable with organic-N, and the sulphur requirement approximates 0.027 × Kjeldahl-N.

When sulphur is present in excess of this requirement, the excess relates to inorganic sulphate in the herbage and its presence indicates that sulphate supply by uptake proceeds at an adequate rate for normal growth.

It is only when total-sulphur assay in the herbage yields values lower than 0.027 × Kjeldahl-N that a true condition of S-deficiency occurs. Features of this condition are the absence of inorganic sulphate in the herbage or its presence in amounts lower than 0.01 gram atoms S per kilogram dry weight,

chlorosis, and reduced utilisation of nitrogen in protein formation relative to total nitrogen assimilation.

A check is made on the frequency of sulphur shortage in field-grown herbage by the use of information from several sources. No conclusive evidence is obtained that inadequate sulphur supply was of significant frequency in the pastures investigated.

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Plant and Soil XIII, no. 3 November 1960

THE INFLUENCE OF WATER SUPPLY ON THE GROWTH AND PHOSPHORUS UPTAKE OF ITALIAN RYEGRASS AND WHITE CLOVER IN POT CULTURE

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INTRODUCTION

In using pot culture to compare the efficiency of fertilizers, it is necessary to have a technique such that differences of 10 per cent or less in the yield of dry matter or fertilizer uptake are statistically significant ($P = 0{\cdot}05$). The ideal is to eliminate all variables which affect plant growth, apart from the fertilizer treatments. In practice this is not possible. The chief variables apart from the fertilizer treatments are the relative positions of replicate pots on a bench or trolley and the moisture content of the soil.

It is usual for a row of pots running lengthwise on a trolley to form one replicate of all the treatments, which are randomised within the row to permit a statistical analysis. The variations between replicates are allowed for in the analysis of variance. Variations within a block other than those due to treatments form the experimental error. As the end-of-row positions may well provide a different environment from central positions, randomisations are rejected or modified if obviously biased in respect of any treatments. [Our earlier work had shown clearly that the extent of environmental differences depended on the degree to which the pots were crowded. Overcrowding was always avoided as far as possible].

The control of soil moisture, if loss of nutrients by leaching is to be excluded, appears to be limited to two methods:

(i) the use of double-walled pots with the inner porous to permit the maintenance of constant soil-moisture conditions (Richards and Blood [8])

(ii) the use of glazed single-walled pots with frequent checking of the moisture content of the soil by weighing and restoring to a predetermined level.

Theoretically the first appears to be more satisfactory, but there is a possibility that the growing conditions may be more natural if the plants undergo some variations in water-stress (Owen and Watson [6]). As a high degree of uniformity of porosity is required the manufacture of double-walled pots is expensive.

In practice single-walled glazed pots are in general use. The practical difficulty is the maintenance of standard moisture conditions. The method used at Jealott's Hill has been to make the average moisture content of the soil in the pots approximate to that at field capacity (Veihmeyer and Hendrickson [9]) at the beginning of an experiment and to check this by weighing all pots at least once each week. Allowances are not made for the increasing weight of plant material as no satisfactory method is known. This, however, is small compared with the weight of water in each pot and rises gradually to become about 4 per cent of the total weight of water in the pot at the time of cutting. The amount of water applied daily is decided by weighing each day about 5 per cent of the pots at random and using the mean deficit figure for all treatments. If the weekly weighings indicate that certain pots are using appreciably more or less water than the mean they are treated individually each day. We have found that the pots where the plants grew more rapidly had a greater daily water deficit.

From time to time considerable variations have been observed in the yields from the replicates of a single treatment. After considering the possible causes, the most probable seemed to be variations in the moisture content of the soil as, at the weekly checks, a variation of $\pm$ 5 to 7 per cent of the initial moisture content of the soil was not unusual. As no information was available about the effects of such variations on dry-matter production and nutrient uptake in pot culture with ryegrass and white clover, some experiments were run parallel with work on phosphorus fertilizers in 1953 and 1954.

EXPERIMENTAL PROCEDURE

Into glazed earthenware pots – 6″ high and $5\frac{1}{8}$″ diameter (both internal) and with no drainage hole – 200 g of silver sand was placed followed by 1,200 g of air-dry grassland soil which had passed a square hole $\frac{1}{4}$″ (6.25-mm) sieve. The soil was a clay loam (U.S.A. Soil Survey Manual [12]) derived from the Lower Oolite limestone pH 7·6, organic matter content 5·4% (Walkley and Black [11]) and moderately low in available phosphorus and potassium.

89·6 mg of P_2O_5 (as 20% superphosphate) were mixed with a further 500 g of soil which was placed on that already in the pot. This method was to simulate field practice *as far as possible* where phosphorus for a ley would be harrowed into the surface. To the surface of the soil in each pot 334·4 mg of potassium sulphate and 334·4 mg of calcium nitrate (as anhydrous salt) were applied in solution. The average soil moisture was then raised to 43 g/100 g oven-dry soil by pouring water down a tube 1″ in diameter placed vertically in the centre of the pot. Italian ryegrass or white clover (S. 100) was sown in the moist soil and covered with 100 g of finer air-dry soil – which had passed a round hole 2-mm sieve.

After the seeds had germinated, all pots were weighed daily at 9.00 a.m. and the water content brought up to the initial value *i.e.* the equivalent of 43 g/100 g oven-dry soil, by pouring water down the central tube. In addition to this sub-irrigation, the equivalent of an $\frac{1}{2}$″ of rain was applied each week to the soil surface ($\frac{1}{4}$″ on Mondays and $\frac{1}{4}$″ on Thursdays). This corresponded to the average weekly rainfall at Jealott's Hill. On these two days correspondingly less water was applied by sub-irrigation.

When the plants were growing well, the following water regimes were established by adding more water to some pots and allowing others to dry partially – 21, 25, 33·3, 43, 54 and 66·6 g water/100 g oven-dry soil *. 33·3 g water/100 g oven-dry soil corresponds with the moisture content at field capacity (not, of course, subjected to the same tension). The lowest water level – 21 g – was appreciably above the wilting percentage (*ca* 15 g water/100 g oven-dry soil). As already explained, it is only possible to quote average moisture figures as a moisture gradient in each pot is unavoidable whatever methods of watering are used. There were 4 replicates of each watering level.

Both the grass and the clover were cut a number of times about half an inch above soil level. 334.4 mg of anhydrous calcium nitrate per pot was given to the Italian ryegrass after each cut followed by another 334.4 mg two weeks later. No nitrogen was applied to the clover after sowing. Both crops had 167.2 mg of potassium sulphate after each cut and the same amount two weeks later. Both salts were applied in solution.

RESULTS

The full data are given for the final year in the Appendix, these results being in agreement with the earlier work. The relationship

* 17·5, 20, 25, 30, 35 and 40% on moist-soil basis *i.e.* g water /100 g moist soil.

between total dry-matter production, total P_2O_5 uptake, and the watering levels are illustrated graphically for both crops in Figures 1 and 2.

With Italian ryegrass, dry-matter production increased as the daily supply of readily available water increased with a tendency

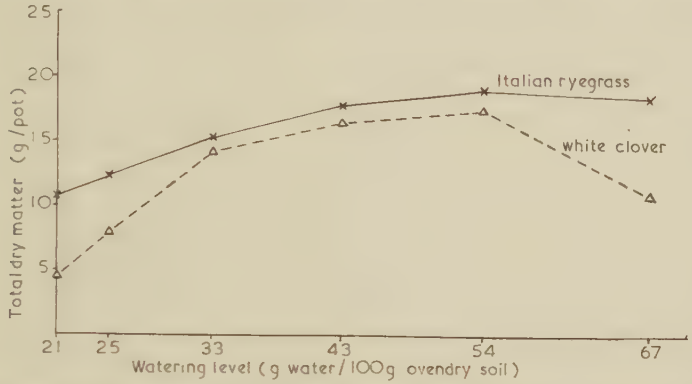

Fig. 1. Relationship between watering level and total dry-matter production of Italian ryegrass and white clover.

to a very slight fall-off at the highest watering level where the soil was temporarily saturated each morning. The behaviour of the white clover with the same watering levels was very different. Under dry conditions, where relatively little water at low tension was available daily, the clover grew much more slowly than Italian ryegrass although the clover pots always had considerably more

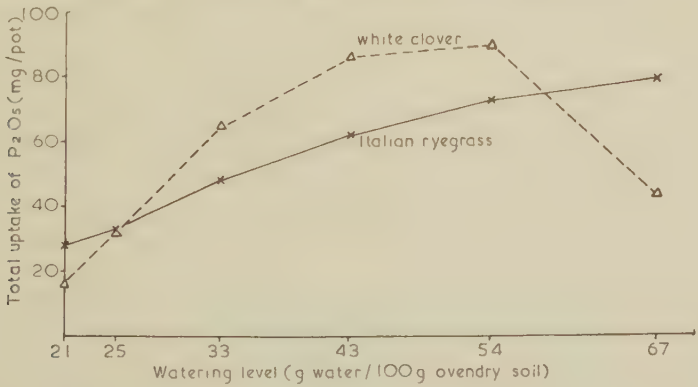

Fig. 2. Relationship between watering level and total P_2O_5 uptake by Italian ryegrass and white clover.

water at the end of each 24 hour period and did not approach the wilting percentage as did the grass. When the average water content was raised to well above the field-capacity figure each morning (43 and 54 g water/100 g oven-dry soil) dry-weight production of grass and clover were very similar. In the same period the clover produced 17·44 g and the grass 18·96 g at the 54-g watering level. Where still more water was added daily, so that the soil was temporarily saturated, the growth rate of clover was greatly reduced, as compared with a slight reduction with the ryegrass.

At the maximum level of dry-matter production of white clover, the water loss was generally about half as great as for the same weight of Italian ryegrass. This is illustrated by Figure 3, where the loss of water for each treatment on three days before a cut of Italian ryegrass and white clover is plotted against the mean yields of dry matter. The cut was taken at a period of vigorous growth in summer. This somewhat surprising result is discussed later in the paper.

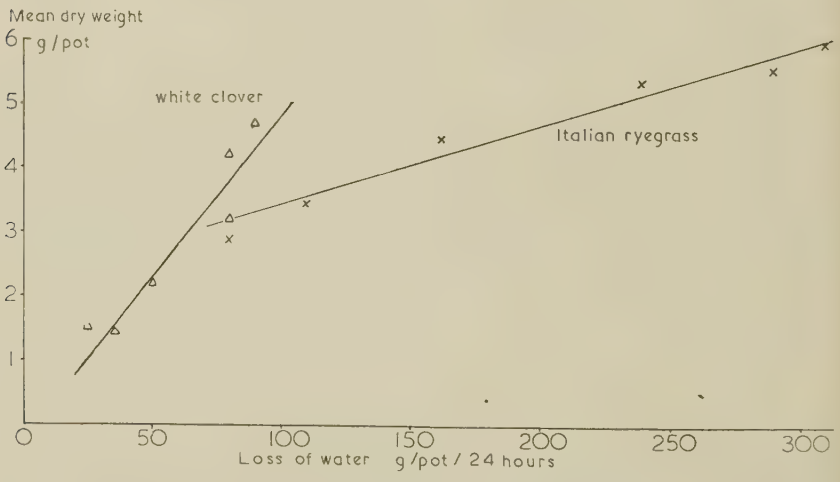

Fig. 3. Relationship between loss of water and mean dry weight of plants three days after loss-of-water measurement.

The dry-matter and P_2O_5-uptake response curves appear superficially to be the same but the highest yield of white clover was 3·8 times the lowest and the perennial ryegrass 1·7, whereas the highest uptake of P_2O_5 by the white clover was 5·7 times the lowest and the perennial ryegrass 2·9. It is rather remarkable that not only

was there a response in dry weight with increase in available water, but also a very considerable increase in the percentage of P_2O_5. The only exceptions were in the last cut of both crops (see Appendix). The absence of an increase there is not surprising since the soil was fairly low in phosphorus initially and was known from earlier work to have strong fixing properties so that, at the higher rates of removal in this experiment, much of the initial dressing would have been taken up in the earlier cuts.

DISCUSSION

The principal object of this investigation was to get information about the effects of the watering procedure in pot-culture experiments on the growth and uptake of phosphorus by Italian ryegrass and white clover. Very marked effects have been obtained. Whilst no account has been found in the literature of strictly comparable experiments, various investigations both in the field and in pot culture are in keeping with our results.

Although conditions in pot culture differ somewhat from those in the field, we have generally found no difference between comparable fertilizer experiments in the field and in pot culture. We have tried to imitate field conditions as, for example, in the attempt to simulate rain already referred to. The more marked response of clover, as compared with Italian ryegrass, to increasing supplies of water agrees with observations made in the field both at Jealott's Hill and elsewhere. Low and Armitage [3] in irrigation experiments found that the maximum growth of clover was obtained where the average soil-moisture tension was lowest (frequently returned to field capacity), and provided there was a good supply of nitrogen, they found perennial ryegrass much less sensitive to water stress than white clover. Van Woudt[12], in a pot experiment with subterranean clover maintained at high and low moisture by weighing at five-day intervals, obtained total yields of 71·70 g and 41·20 g respectively.

At the lowest watering level, whilst the grass reduced the water content to near wilting percentage daily, it was not so with the clover where it always remained well above this level although dry-matter production was very much less with the latter crop. At all watering levels, including those where the dry-matter pro-

duction was nearly the same for both crops, the water content of the soil at 9.00 a.m. was always considerably greater with clover than the corresponding grass treatment. This suggests that white clover can only readily utilize water at low tensions as otherwise dry-matter production should have been greater at the lower watering levels. Water uptake was not limited by root development as there was a good distribution with both crops.

Evidence that plants vary in the ease with which they can utilise water over the same tension ranges is given in the review on soil moisture in relation to plant growth by Veihmeyer and Hendrickson [9].

The sharp reduction in the dry-matter production of clover at the highest watering level may have been due to daily reductions of the oxygen tension in the soil after watering, and to which white clover may be more sensitive than Italian ryegrass owing to the requirements of the root nodules for nitrogen fixation. Unlike the Italian ryegrass the white clover did not receive any nitrogenous fertilizer apart from a small seedbed dressing.

Whilst no reference to poor growth of white clover in wet soil conditions has been found in the literature, a considerable number of instances of restricted growth of white clover during seasons of higher than average rainfall, and other wet conditions have either been seen by or described to the authors.

There is no obvious explanation for the marked difference in transpiration from similar dry weights of white clover and Italian ryegrass where the yields were highest and there was a large water supply *. It is possibly due to the different habits of the plants. Whereas the grass consisted very largely of leaves, much of the dry matter of the clover plants was in the stems and stolons. It is known that the amount of water evaporating from stems is small, the greater portion of transpirational loss being from leaves (Miller [4]). In addition the general arrangement of the clover leaflets would tend to protect the lower layers of leaves from freely transpiring in a way that was not apparent with the ryegrass.

The results on the uptake of phosphorus are in line with various observations in the literature. Orr [5] refers to the work of Klimer

* It is appreciated that the *relative* water losses from the two species in pots may not be the same as in the field where the losses should be about the same from both species according to Penman [7].

and Schmidtt who found from the analysis of hay crops that the species concerned took up less calcium and phosphorus during severe drought. Coleou [1] found "the supply of water in regions of average or low rainfall during the growing period can cause much greater increases in phosphorus content than fertilizing can". In their review of soil moisture in the mineral nutrition of plants, Wadleigh and Richards [10] state that "most experimental evidence shows that for a given level of fertility, decreasing soil moisture supply is associated with a definite increase in nitrogen content of the plant tissue, a definite decrease in the potassium content and a variable effect on the content of phosphorus, calcium and magnesium". Emmert [2], in a pot experiment with tomatoes found "lack of moisture had a decidedly depressing effect on the phosphate concentration in a plant".

It seems possible that the varying mineral composition of grasses and clovers in the course of a growing season may be due *in part* to changes in average soil water stress. With the same total rainfall, for example, in April and July in Southern England, the average water stress to which herbage plants are subjected will be generally considerably greater in the latter month because of the much greater losses by evapotranspiration.

SUMMARY

White clover appears to be much more sensitive, in terms of dry-matter production, to variations of soil moisture than Italian ryegrass. The results show that a variation of 5 in the percentage of water (moist-soil basis) on either side of the field-capacity figure can cause a statistically significant effect in the dry weight of Italian ryegrass in pot culture, although a slightly larger percentage may be necessary for white clover to be affected as standard errors in pot culture with this crop tend to be higher.

With an adequate supply of soil phosphorus the greater the amount of readily available water the greater the percentage of P_2O_5 in Italian ryegrass; with white clover it rises to a maximum then falls off under very wet conditions. Statistically significant differences in percentage P_2O_5 for both crops can be obtained whit those variations in the water supply which caused significant differences in dry weight.

ACKNOWLEDGMENT

The authors wish to thank Mr. P. A. Collier for the statistical analysis.

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For **APPENDIX** see p. 251 and 252.

APPENDIX, TABLE 1

Mean dry weight, P_2O_5 percentage and uptake of **Italian ryegrass**

Watering level g water 100 g oven-dry soil	Dry weights, g					Mean P_2O_5 percentage				P_2O_5 uptake, mg				
	Cut 1	Cut 2	Cut 3	Cut 4	Total Cuts1-4	Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4	Total Cuts1-4
21	3.40	2.86	2.48	1.99	10.73	0.21	0.25	0.33	0.26	7.1	7.2	8.3	5.0	27.6
25	3.50	3.47	3.17	2.16	12.30	0.23	0.28	0.32	0.24	8.1	9.7	10.3	5.2	33.2
33.3	4.03	4.46	3.58	3.13	15.21	0.30	0.35	0.38	0.22	12.0	15.6	13.8	6.9	48.2
43	4.62	5.31	3.90	3.87	17.69	0.35	0.40	0.41	0.22	16.2	20.9	16.1	8.7	61.9
54	4.43	5.51	4.04	4.99	18.96	0.39	0.44	0.45	0.26	17.4	24.1	18.2	12.9	72.6
66.6	3.91	5.92	4.17	4.50	18.50	0.46	0.50	0.47	0.28	18.2	29.2	19.6	12.6	79.6
Standard error (single pot)	± 0.26	± 0.36	0.24	0.58	± 0.72	± 0.02	± 0.03	± 0.02	± 0.03	± 1.2	± 1.4	± 1.0	± 1.0	± 2.9
Coefficient of variation	6.6%	7.8%	6.7%	16.8%	4.6%	5.5%	7.3%	5.4%	11.8%	8.9%	7.7%	7.0%	11.8%	5.3%
Significant difference between treatment means														
$P = 0.05$	0.40	0.54	0.36	0.87	1.08	0.03	0.04	0.03	Not	1.8	2.1	1.5	1.5	4.3
$P = 0.01$	0.55	0.74	0.50	1.21	1.50	0.04	0.06	0.04	Sig.	2.4	2.8	2.1	2.1	6.0

APPENDIX, TABLE 2

Mean dry weight, P_2O_5 percentage and uptake of **white clover**											
Watering level g water/100 g oven-dry soil	Dry weights, g				Mean P_2O_5 percentage			P_2O_5 uptake, mg			
	Cut 1	Cut 2	Cut 3	Total Cuts 1-3	Cut 1	Cut 2	Cut 3	Cut 1	Cut 2	Cut 3	Total Cuts 1-3
21	1.51	0.93	2.12	4.56	0.25	0.57	0.29	4.0	5.5	6.2	15.7
25	2.20	2.17	3.56	7.93	0.36	0.61	0.30	7.9	13.4	10.3	31.6
33.3	3.18	3.86	7.12	14.16	0.57	0.69	0.29	18.2	26.6	20.6	65.4
43	4.68	3.88	7.85	16.40	0.64	0.82	0.31	29.9	31.7	24.0	85.7
54	4.16	4.16	9.12	17.44	0.66	0.81	0.33	27.0	33.6	29.5	90.1
66.6	1.44	2.40	6.98	10.82	0.44	0.71	0.30	6.3	16.9	20.6	43.8
Standard error (single pot)	±0.71	±0.49	±1.15	±1.86	±0.08	±0.08	±0.03	±3.4	±3.1	±3.6	±8.2
Coefficient of variation	24.9 %	16.7 %	18.7 %	15.6 %	16.2 %	10.9 %	9.7 %	21.8 %	14.7 %	19.3 %	14.8 %
Significant difference between treatment means											
$P = 0.05$	1.07	0.73	1.73	2.80	0.12	0.12	Not	5.1	4.7	5.4	12.3
$P = 0.01$	1.49	1.01	2.39	3.87	0.17	0.16	Sig.	7.1	6.5	7.5	17.0

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THE CONTACT-EXCHANGE THEORY AMENDED *

by J. V. LAGERWERFF **

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INTRODUCTION

In a previous paper [7] experimental results were presented relative to the yield and mineral composition of red kidney beans grown on a number of synthetic substrates which, while the ions present were in electrochemical equilibrium throughout, differed with respect to the presence or absence of adsorbed ions. It appeared that under the prevailling conditions ions in the adsorbed phase exerted no influence different from that of freely diffusing ions on the test plants. A theory was put forward, based on the thermodynamic description of equilibrium, predicting this outcome. The viewpoint was expressed that as a result of the equilibrium *** the concentration (activity) of the cations adsorbed at the root exchange sites is virtually the same regardless of whether the roots are in direct contact with the diffuse layer of counter ions (the electric double-layer) extending from the exchanger surface, or with the ambient ionic equilibrium solution.

Earlier studies on the same subject, and on the depletion of ions from roots in an environment with or without exchangeably adsorbed ions, have led to the "contact-exchange" theory [4 5]. It at-

* Contribution from the U.S. Salinity Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U.S. Department of Agriculture, Riverside, California, in cooperation with the 17 Western States and Hawaii.

** Soil Scientist

*** Where the existence of an ionic equilibrium is postulated in systems involving ion-absorbing biological materials, this equilibrium cannot be but approximate because of the existence of a force responsible for the ion movement. For the sake of the present argument equilibrium conditions are assumed to be characterized by the infinitesimal magnitude of this force. This assumption in no way determines the rate at which the ions move because of the latter's dependence on the diffusivity of the medium.

tempts to explain the observed faster rate of cation uptake by, or cation depletion of plant roots when in contact with cations adsorbed on an exchanger, as compared with the rate of the same processes involving freely diffusing cations. The cation-exchange theory postulates the occurrence of ion transfer by surface-migration whenever the "oscillation volumes" of the ions adsorbed on the surfaces of plant root and exchanger, *i.e.* the respective double layers, are overlapping. The process of ion transfer by contact-exchange, which supposedly takes place without the aid of the soil solution, and, therefore, independent of the water content of the soil or the transpiration of the plant, is considered to be endowed with a greater rate than ion transfer processes involving free diffusion through a solution followed by adsorption at the root exchange sites.

Both theories view the initial entry of ions into plant roots as a process subject to physicochemical considerations. It should be emphatically denied that, in doing so, ion uptake is considered to be governed by physicochemical laws. The prevalence is fully recognized of a metabolic phase of ion uptake. Yet, based on numerous independent observations, the contention is upheld that ions to be metabolized are being drawn from the pool of those that have entered the plant root by diffusion and/or adsorption, processes which are of an exact physicochemical subject matter. Also, both theories accept, at least for mono-cationic systems involving unsaturated root exchange sites, that the rate of the subsequent accumulation of the cations is a function of their concentration at the exchange sites. Nonetheless, the interpretations put forward by the two theories to account for, and validate the different experimental results seem to be in flagrant conflict. It is the purpose of this paper to show that this impression is only incidental, and is caused mainly by a disregard for the exact nature of the conditions under which it is permissible to apply the current contact-exchange theory. In the following, the conditions will be investigated pertinent to the application of the thermodynamic and contact-exchange theories to the initial uptake of ions by plant roots. The following points will be emphasized: ionic equilibrium, ionic concentrations, and contact between the double-layers of ions adsorbed on exchanger and root surfaces.

EXPERIMENTAL DETAILS

1. *Design*

Specimens of dwarf red kidney beans were subjected to six different treatments. The beans were grown either on sand, or on mixtures of sand and particles of cation-exchange resin, and irrigated with three different solutions. In addition to constant amounts of K_2SO_4, NH_4NO_3, K_2HPO_4, H_3BO_3, $MnCl_2$, and Fe-citrate, the solutions contained variable amounts of NaCl, $CaCl_2$, and $MgCl_2$, as indicated in Table 1. The latter three salts were

TABLE 1

Composition of the irrigation solution, in me per liter, as a function of the sodium-adsorption ratio, $Na^+/\sqrt{Ca^{++} + Mg^{++}}$ mmoles$^{\frac{1}{2}}$ liter$^{-\frac{1}{2}}$ as the chlorides.*			
Sodium-adsorption ratio	Na	Ca	Mg
2.7	9.0	15.0	5.0
19.3	22.1	1.9	0.8
51.2	23.7	0.3	0.1

* In addition, per liter of solution: 3me K_2SO_4, 2 me NH_4NO_3, 0.5 me KH_2PO_4, 0.5 mg B as H_3BO_3, 0.5 mg Mn as $MnCl_2.4H_2O$, and Fe as Fe-citrate administered twice weekly at a rate of 1 mg Fe per liter of solution. The three solutions were isotonic at 1.3 atm of osmotic pressure.

added in such a manner that three values were established for the sodium-adsorption ratio, $Na^+/\sqrt{Ca^{++} + Mg^{++}}$, of the solutions, *viz* 2.7, 19.3, and 51.2 mmoles$^{\frac{1}{2}}$ $l^{-\frac{1}{2}}$ in terms of concentrations, or 3.5, 24.3, and 63.7 in terms of activities. The calcium and magnesium salts were added in proportions of 3 : 1. Prior to planting, the sand-resin mixtures were equilibrated with the solutions employed for irrigation. The experiment was carried out in triplicate.

2. *Materials*

Upon being nurtured from seed to seedling on cheesecloth stretched above a four-fold dilution of Hoagland's nutrient solution, the bean plants were transferred to the sand and sand-resin substrates, and permitted to grow to maturity under Southern California greenhouse conditions during the last three months of 1958. The sand used was an acid-washed coarse silica sand as used for glass manufacturing, purified from heavy-metal compounds by being passed under powerful electromagnets, and freed from finer fractions by the sedimentation method. The cation-exchange resin used was Amberlite IR-120 with an exchange capacity of 0.9 me per g. The cation-exchange capacity of the sand-resin mixtures amounted to 8 me per 100 g.

3. *Methods*

The substrates of sand and of sand mixed with resin were irrigated with about one-half gallon of nutrient solution once every hour. In addition, in one instance involving solution with a sodium-adsorption ratio of 19.3, the

irrigation was performed once every 24 hours. Mechanically, the irrigation was effected by forcing the solution upward from five-gallon storage reservoirs through Tygon tubing to irrigators of the same material placed on the surface of the solid substrate. Power was supplied by a pressure pump connected with a time clock. Due to the large pores and low waterholding capacity of the substrate, the irrigation solution percolated rapidly, and was recollected through a hole in the convex bottom of the culture jar into the proper storage reservoir. The change in the composition of the culture solution as a result of nutrient uptake was limited to a minimum by replacing the entire bulk nutrient solution in the storage reservoirs once every week, or more often when necessary.

After recording the yield at the end of the growth period, the cationic composition was determined on representative samples of leaves and stems. The chemical analyses were carried out on wet (acid) digests of the dried and ground plant material. Sodium and potassium were determined using the Beckman model-41 direct-reading flamephotometer. Calcium and magnesium were measured collectively by means of the versenate-titration method, modified to include the prior removal of interfering elements by means of zirconium oxychloride [10].

RESULTS AND DISCUSSION

The data obtained are presented in Table 2. Except in one instance, the results indicate the absence of significant differences in fresh weight of the pods and mineral composition of leaves and stems of the 3-months old dwarf red kidney bean plants when grown in the absence of adsorbed cations as compared with their presence.

Prior to speculating upon the significance of these results, at least two crucial points have to be considered. The first one pertains to the argument that at the high electrolyte concentrations employed no differences in ion accumulation due to contact-exchange may be expected. In order to throw light on this question, the experimental design provided for one instance in which the irrigation with the nutrient solution was carried out once every 24 hours. Valid comparisons as to yield and mineral composition can be made between plants grown under these conditions, and plants grown under conditions that are similar but for the fact that the irrigations were performed at the usual hourly intervals. In Table 2, the cases referred to are those corresponding with a sodium-adsorption ratio of *19.3* and 19.3 $mmoles^{\frac{1}{2}}\ l^{-\frac{1}{2}}$, respectively. Whereas the cultures irrigated at hourly intervals do not, those

TABLE 2

Average of triplicate values pertaining to the fresh weight of the pods, and the mineral composition of the leaves and stems of three-month of old dwarf red-kidney beans as a function of the sodium-adsorption ratio of the solution used to irrigate the sand in which the plants were cultured in the presence and absence of Amberlite IR-120 cation exchange resin (8 me per 100 g of mixture). The ions in solution and on the resin were in electrochemical equilibrium in all cases but the one corresponding to a sodium-adsorption ratio of *19.3*.

Sodium-adsorption ratio	Resin	Mineral composition, me per 100 g dry weight						Fresh weight of pods, g
		Na		K		Ca + Mg		
		Leaves	Stems	Leaves	Stems	Leaves	Stems	
2.7	no	2.3	1.5	7.8	48	192	35	42
	yes	2.4	1.7	7.6	49	190	34	43
19.3	no	2.3	4.5	13	42	87	19	33
	yes	2.4	4.6	12	43	83	18	32
51.2	no	3.7	13	17	56	33	13	14
	yes	3.5	12	18	53	33	15	15
*19.3**	no	4.2	6.8	16	58	115	21	14
	yes	3.0	8.4	12	78	135	28	21

* Irrigated at 24-hour intervals, in contrast to the one-hour intervals at which all other treatments were irrigated.

irrigated at 24-hour intervals do show differences in growth and composition due to the presence or absence of adsorbed cations. This finding is interpreted as indicative of a state of non-equilibrium between the ions in solution and in adsorbed form brought about by the increase in time lapse between irrigations in conjunction with the extremely low waterholding capacity of the substrate. These "non-equilibrium" conditions are believed to be characteristic of the fact that the rate of ionic diffusion in the solution phase around the roots and adjacent solid phase under influence of water uptake by the plant, increasing the moisture tension, was gradually reduced to the point where the amount of ions reaching the root exchange sites per unit of time fell behind their rate of accumulation (cf Ref.[6]). In contrast, the amount of adsorbed, surface-migrating ions reaching the root exchange sites per unit of time, in spite of their diminishing rate of transfer under increasing moisture tension, by sheer numerical superiority could better meet the demands made by the plant. This interpretation, which is not in conflict with the contact-exchange theory, may serve to dispell doubt as

to whether ionic concentrations were too high for contact effect to show up.

The second point, not independent from the first one, deals with the extent of contact between the ions adsorbed on the exchanger and plant root surfaces. According to the contact-exchange theory, this contact should be sufficiently intimate to enable ions adsorbed on an exchanger surface to contribute directly to the mineral nutrition of the plant by ion exchange between the contacting double-layers, without the intervention of the free, intermicellar solution. As has been stated earlier [7], it is felt that it will prove to be well-nigh impossible to devise and execute a critical experiment purporting to ascertain the exact identity of the root environment without at the same time adversely affecting the test plant involved. Without undue speculation it may be deduced, for instance, that when roots are strapped to, and even weighed down on strips of exchange resin so firmly as to reduce the interstratification of free solution to a minimum, root injury is being inflicted, leading to wider pathways for ion entry by simple diffusion (cf Ref.[2]). At another place [9], disregarding normal cation exchange under the influence of hydrogen ions and impurities, it has been surmised that the presence of cation-saturated exchange resins in contact with water to which no salts had been added would bring about a root environment characterized by the presence of adsorbed ions only. The obvious invalidity of this assumption once more points to the difficulty of obtaining any experimental verification of contact exchange of ions. While the point raised, therefore, is considered to be beyond the reach of current experimental techniques, it may, belatedly, be asked whether the it is of any significance.

The answer to the latest question depends on conditions that are determined by the purpose of the investigation. If one wishes to compare the behavior of the ions in two different systems with respect to a third system, the imperative condition is the equalization of the thermodynamic state of the ions throughout the first two systems, *i.e.* the establishment of electrochemical equilibrium between the ions in all phases of both systems. In the present study the ions at the exchanger surface in conjunction with those at the root exchange sites active in ion transfer on the one hand, and the ions in the free solution in conjunction with similar exchange sites on the other hand have been compared with respect

to the accumulatory activity of the root cells. The significance of the results, presented in Table 2, is the fact that in corroborating with those obtained previously [7], the evidence has been strengthened pertinent to the viewpoint 1) that the sustained existence of electrochemical equilibrium between the ions adsorbed at the surface of exchanger and plant root, and those occurring in free solution, constitutes the absolute prerequisite for any comparative ion-uptake study possessing a meaningful intent, and 2) that, under these circumstances, adsorbed ions exert no influence different from that of freely diffusing ions on the plants grown.

The above line of thought may serve to introduce the main objections that it is felt should be raised against the present status of the contact-exchange theory. These objections are twofold and deal with the neglect by the contact-exchange theory to take into consideration either parameter in the right hand term of the following conventional expression describing the process of translocation of ions:

$$r = k\frac{d\bar{\mu}}{dx} \qquad (1)$$

The symbol r indicates the rate of translocation, the constant k pertains to the diffusivity of the medium for ions, and $d\bar{\mu}/dx$ represents the gradient of the electrochemical potential, $\bar{\mu}$, with respect to the distance, x, or the driving force. In the first place, the fact is ignored that the diffusivity for ions of water occurring under the constraint of the electric field forces extending from the exchanger surface is lower than that of unbound water [8]. The impact of this interaction on the rate of ion transfer depends, of course, on the magnitude of the ratio $d\bar{\mu}/dx$. The discrepancy between the non-equilibrium data reported in this paper, and the outcome of previous experiments of a similar nature [1] points to their incomparability caused not only by probable differences in the value for $d\bar{\mu}/dx$, but also by the widely different moisture regimes observed, resulting in unequal diffusivities of the culture media for ions.

In the second place, the contact-exchange theory is heedless of the value for $d\bar{\mu}/dx$, and, thereby, of the thermodynamic state of the ions of any one species present in the various phases of the root environment. Illustrative of this viewpoint is the rather precocious statement [5] that: "in order to ascertain the presence of contact

intake it must be shown that plants accumulate more ions from clay suspensions than from their carbonic acid extracts". The extracts in no way representing solutions in equilibrium with the suspensions, these systems are incomparable with respect to ion uptake by plants. Elsewhere [4], claiming the occurrence of equilibrium, the unlikely coexistence is proposed of conditions characterized by an equal amount of net transfer of alkali metal ions by solution-exchange and by contact-exchange between two different exchangers (in agreement with the thermodynamic concept), with a "greatly accelerated" rate of transfer by contact-exchange. The impression is thus created that, when applied to ion uptake by plant roots from suspensions and solutions in which the ions occur in a state of equilibrium, the contact-exchange theory in its present form is in conflict with basic thermodynamics. In the following, this point will be further investigated.

The contact-exchange theory, by claiming that the rate of ion transfer by surface-migration is faster than by diffusion and adsorption, tacitly holds that the concentration or activity of ions adsorbed at the exchange sites of an unsaturated root surface is greater when the root surface is in contact with the adsorbed phase extending from the exchanger surface, than in the corresponding case involving a "comparable" solution. For the chemical activity, a, of the cations of a single monovalent species, one could describe this situations as follows:

$$a^+{}_{r,\,e} > a^+{}_{r,\,s} \tag{2}$$

where the subscripts indicate the exchange sites of the roots r in contact with the ions at the exchanger surface e, or in solution s. By applying Nernst's equation separately to each of the two terms constituting the above inequality (2), one obtains the following expressions:

$$a^+{}_{r,\,e} = a_e{}^+ . e^{y_e - y_r} \tag{3}$$

$$a^+{}_{r,\,s} = a_s{}^+ . e^{-y_r} \tag{3a}$$

In the exponential, the symbol y represents the ratio $\varepsilon\psi/RT$, where ε is the electronic charge, ψ the electric potential at the location of the ions, R the universal gas constant, and T the absolute temperature. According to convention, the electric potential of the ions in the solution is assumed to be zero. Further development yields:

$$RT\ln(a_r^+)_e + \varepsilon\psi_r = RT\ln a_e^+ + \varepsilon\psi_e \quad (4)$$

$$RT\ln(a_r^+)_s + \varepsilon\psi_r = RT\ln a_s^+ \quad (4a)$$

In the terminology used by Guggenheim [3], expression (4) describes the equality of the electrochemical potential of the cations concerned that occur adsorbed at the exchange sites of the roots, and at the exchanger surface. Equation (4a) describes a similar situation for these cations at the root exchange sites in contact with the ambient solution. The two equations can be contracted to:

$$\bar{\mu}^+_{r,\,e} = \bar{\mu}_e^+ \quad (4')$$

$$\bar{\mu}^+_{r,\,s} = \bar{\mu}_s^+ \quad (4a')$$

From equation (2) it follows, according to the contact-exchange theory, that the following order exists between the equations named:

(3) > (3a), and (4) > (4a), or, (4') > (4a'), *i.e.*:

$$\bar{\mu}^+_{r,\,e} = \bar{\mu}^+_e > \bar{\mu}^+_{r,\,s} = \bar{\mu}_s^+ \quad (5)$$

Under conditions of electrochemical equilibrium between the ions adsorbed at the root exchange sites and at the exchanger surface, and those freely diffusing in solution, such as have been claimed in the case of one recent experiment [2], one has to write:

$$\bar{\mu}^+_{r,\,e} = \bar{\mu}^+_e = \bar{\mu}^+_{r,\,s} = \bar{\mu}^+_s \quad (6)$$

In the paper just quoted it has been proposed in essence to identify the conditions described by the incongruous equations (5) and (6). From their incompatability it follows that as long as the contact-exchange theory in its present form is linked to conditions of equilibrium, basic theory is violated. In fact, the Second Law of thermodynamics appears to be placed in jeopardy. This becomes clear if one comprehends the consequences of the contact-exchange theory where it leads to the deduction that the concentration (activity) of the cations adsorbed at the exchange sites of unsaturated root surfaces would be higher in a clay suspension than in the corresponding equilibrium solution. According to Nernst's equation, when arrayed in the proper manner and linked in one circuit, an electric potential difference may be registered between two such identical roots, pointing to the possibility that work might be gained from a system in thermodynamic equilibrium. Obviously, this cannot be correct.

Except in the one instance just discussed, ion uptake or depletion from or by a clay suspension have been compared with that from or by a "comparable" or "corresponding" solution, the composition of which was, from the outset on, not the result of a deliberate equilibration [4 5]. As results of non-equilibrium processes, the reported observations are fully acceptable.

In Table 3, a number of possible conditions pertinent to the occurrence of equilibrium and non-equilibrium between the ions present have been presented, and the extent to which they are covered by the contact-exchange theory and the thermodynamic concept, each, has been indicated. Far from being incompatible, it is held that the two viewpoints, provided they are applied under fitting circumstances, each may explain component phenomena

TABLE 3

Six theoretical cases illustrating the rate of cation uptake by plant roots at any one moment from the exchanger phase (u_e) relative to that from the solution phase (u_s) as a function of the relation between the rate of cation transfer from the surroundings to the root exchange sites by surface migration (r_{sm}) or diffusion and adsorption (r_{da}) and the maximum rate of inward cation transfer from the exchange sites to inner cell areas (r_{ia}) at that moment. The prevalence of uniform growing conditions, and the presence of cations of a single species is assumed.

Case	$\frac{r_{sm}}{r_{ia}}$	$\frac{r_{da}}{r_{ia}}$	Equilibrium *	$\frac{a_{r,\,e}}{a_{r,\,s}} = \frac{u_e}{u_s}$ **	Remarks
A	> 1	> 1	yes	1	Cases covered by thermodynamic concept
A′	$\gg 1$	> 1	yes	1	
B	< 1	< 1	yes	1	Same, and only condition proper for experimental proof
B′	< 1	$\ll 1$	no	> 1	Cases covered by present contact-exchange theory
C	> 1	< 1	no	> 1	
D	< 1	> 1	no	< 1	Case ignored

The symbols $<$ and $>$ indicate "smaller than" and "greater than" by a similar factor, whereas the symbols $\ll$ and $\gg$ represent twice the value of this factor.

* See footnote on page 253. Approximate equilibrium between the ions occurring in exchanger phase, in solution phase, and on root exchange sites involved at any one moment in ion transfer.

** For $a_{r,\,e}$ and $a_{r,\,s}$ see equation (2). The equality follows from the postulated monocationic nature of the systems and uniform growing conditions in all phases of the growth medium, and does not imply that cation uptake, in its entirety, is a process subject to physicochemical considerations.

dealing with the initial entry of ions into plant roots. As stated previously [7], it is under conditions of equilibrium that the ionic environment of the plant root, while in all likelihood consisting of ions in both the adsorbed and solution phases, is entirely characterized by the ionic composition of the solution phase. This concept would make available the large body of data collected by means of solution-culture studies of plants for interpretative use in certain types of soil-culture work.

The probability of contact-exchange in no way being refuted by the thermodynamic explanation, it is felt that the contact-exchange theory, having first formulated this phenomenon in a mechanical sense, should be amended. Henceforth, it should recognize that, depending on the diffusivity of the medium for, and the thermodynamic state of the ions in the suspension and solution phases of the culture system, the uptake from the suspension phase may be either faster than, equal to, or slower than that from the solution phase.

SUMMARY

Experimental data have been presented showing that over a three-month period the rate of cation uptake by kidney bean plants from a substrate containing both adsorbed cations and cations in solution is equal to that from a solution only, provided the ions in the solution are in thermodynamic equilibrium with those in the adsorbed from. These data, in conjunction with presentations in the literature which would seem to validate the "contact-exchange" theory, have been critically analyzed. It has been concluded that while probably correct in assuming contact-exchange between ions adsorbed at or near the surfaces of plant root and exchanger, the contact-exchange theory should be amended so as to recognize that the rate at which this exchange proceeds is a function of the diffusivity of the medium for, and the thermodynamic state of the ions in the various phases of the nutritional environment of the roots. The faster rate of ion-uptake by contact-effect (surface-migration) than by diffusion and adsorption, as frequently reported, is considered to be typical of unequilibrated ionic conditions, whereas a corresponding equal rate of uptake is held to be accounted for by assuming the existence of thermodynamic equilibrium between the ions in the various phases of the system. This equilibrium, naturally, cannot be but approximate because of the occurrence of a driving force prompting locomotion of ions. Under the equilibrium conditions thus specified, the ionic environment of the plant root is entirely characterized by the ionic composition of the solution phase. This deduction may serve to show that under certain conditions the same interpretative value may be placed on the outcome of soil- and solution-culture studies.

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THE INFLUENCE OF CARBON DIOXIDE ON SYMBIOTIC NITROGEN FIXATION

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In a previous investigation [12] it was found that the roots of red-clover plants growing on acid sandy soils developed nodules only poorly or not at all. Treatment of the soil with calcium carbonate or sterilized extracts of yeast or Rhizobium cells resulted in much improved nodulation, nitrogen fixation and growth of the clover plants. If the similar effects produced by the three treatments are due to a common factor it is possible that this is carbon dioxide in the case of calcium carbonate and 4-C dicarboxylic acids or other intermediates of the Krebs cycle in the case of yeast or Rhizobium extracts.

The importance of carbon dioxide as a growth factor for heterotrophic micro-organisms, the leaves of succulent plants in the dark, and plant roots has been demonstrated by a number of workers [4 7 8 9 13 14 16]. Ajl and Werkman [1 2] found that CO_2 could be replaced by intermediates of the Krebs cycle and related compounds, such as α-alanine, aspartic acid, and glutamic acid, in experiments with *Escherichia coli* and *Aerobacter aerogenes*.

The purpose of the present investigation was to study the effect of the presence of carbon dioxide in the nutrient medium on nodulation and nitrogen fixation in leguminous plants. Red-clover plants growing in culture solution at two different pH values and also in acid soil were used in the experiments. In addition the effect of CO_2 was compared with the effect of sterilized suspensions of *Rhizobium trifolii*. An experiment with pea and bean plants was conducted in culture solution at pH 4.8.

EXPERIMENTAL

Cultures in nutrient solutions

The culture solution employed had the following composition: KH_2PO_4 120 mg, K_2HPO_4 360 mg, $MgSO_4.7H_2O$ 250 mg, $CaSO_4.2H_2O$ 250 mg, NH_4NO_3 30 mg, $MnSO_4.1H_2O$ 1 mg, $ZnSO_4.7H_2O$ 0.25 mg, $CuSO_4.5H_2O$ 0.25 mg, H_3BO_3 0.25 mg, and $Na_2MoO_4.2H_2O$ 0.05 mg per 1 of distilled water. Iron was supplied in the form of EDTA-complex, 20 and 10 mg Fe per l, respectively, in the first two experiments with clover and as ferric chloride, 5 mg Fe per l, in the experiment with peas and beans.

To obtain a reaction of pH 4.8 the same amount of phosphate was supplied but entirely as KH_2PO_4.

Glass containers of 2-l capacity each containing one plant and provided with an aeration tube were used.

Before being transferred to the containers the clover plants were grown for 10 days on sterile, moist sand or on agar slopes, solidified with 0.6% Davis agar, containing 7 ml of the above-mentioned culture solution. The pea and bean plants were similarly grown for 5 days on agar and sterile moist sand, respectively, before being transferred. The plants were inoculated with effective strains of Rhizobium one or two weeks after they had been transferred to the culture vessels. To facilitate the infection the reaction of the culture medium after inoculation was kept for 2 weeks at pH 5.3. Thereafter it was kept at pH 4.8.

The glass containers of the various treatments were placed in alternate rows to avoid localized differences in the CO_2 supply to the leaves, due to aeration of the culture solution, as far as possible. In the third experiment with clover plants and in the experiment with pea and bean plants the rubber covers containing the plants were fitted with glass tubes 45 cm in length and 0.8 cm in diameter to disperse the aeration gasses above the plants. In addition electric fans were used to prevent stagnation of the atmosphere to which the leaves were exposed

Sterilized suspension of Rhizobium cells. This suspension was prepared as described in a previous paper [12].

Analytical methods

Nitrogen was determined according to the Kjeldahl-Lauro method as modified by Yuen and Pollard [17].

Amino acids were determined chromatographically by a quantitative method described in previous papers [10 11].

Non-volatile organic acids were determined chromatographically according to the method of Heitefuss [6] and Wolffgang [15]. For these analyses aliquots of the concentrated nodule extracts, prepared as described earlier [11], were acidified with sulphuric acid and extracted with freshly distilled ether in a Kutscher-Steudel continuous-extraction apparatus for 72 hours. A small amount of water was then added and after the ether had been evaporated off the residual aqueous solution was made up to 1 ml with

H_2O. Aliquots of 0.25 ml were transferred to 50 × 50 cm sheets of Whatman no-1 filter paper and, after drying the spots, 2-directional descending chromatograms were run first with ethanol—ammonia—water (80 : 4 : 16) and then with butanol—formic acid—water (77 : 12 : 11) as the moving phases. The chromatograms were then dried for 15 hours at room temperature in a current of air before being sprayed with a 7.5% solution of glucose in aniline—butanol—ethanol—water (20 : 365 : 175 : 100). The colour was developed by heating the sheets for 45 minutes at 105 C. By this method the separation and identification of the following organic acids from a mixture of the chemically pure compounds were achieved: oxalic, glycolic, malonic, lactic, glyceric, fumaric, succinic, malic, maleic, tartaric, oxalacetic, glutaric, α-ketoglutaric, adipic, citric, cis-aconitic, and nicotinic acids. Pyruvic acid was lost during the procedure. In addition to the above mentioned organic acids the following inorganic acids could be identified: sulphuric, phosphoric, nitric, and hydrochloric acids.

RESULTS

a. *Effect of carbon dioxide and a suspension of sterilized Rhizobium cells on nodulation and nitrogen fixation by inoculated red clover growing in culture solution*

Pots of 2-l capacity filled with the previously mentioned nutrient solution and each containing one plant were used. There were ten pots in each of the following series: *1*) culture solution with a reaction of pH 4.8 and aerated with air free from carbon dioxide, *2*) culture solution with a reaction of pH 4.8 and aerated with air containing approximately four per cent of carbon dioxide, *3*) and *4*) similar to *1* and *2*, except that the reaction of the culture solution was maintained at pH 6.4, *5*) culture solution with a reaction of pH 4.8, to which was added a suspension of killed Rhizobium cells, *6*) as *5* but with a reaction of pH 6.4. The culture solutions of the pots of Series *5* and *6* were aerated with CO_2-free air.

The experiment commenced on December 20th 1958 when plants which had been grown on sterile sand were transplanted to the glass containers. Inoculation with an effective strain of *Rhizobium trifolii* was carried out on January 10th. On this date killed suspensions of Rhizobium were added to the pots of Series *5* and *6*. The reactions of the culture solutions were adjusted daily to their respective initial pH values.

On February 2nd clear differences were observed between plants grown in the absence and in the presence of carbon dioxide in the culture solution. In the absence of carbon dioxide the roots were

somewhat thicker but considerably less branched and the nodules were smaller and fewer in number. The nodules were, in general, restricted to the main roots as compared with their distribution throughout the root system when air containing carbon dioxide was used for aeration. Shoot development was also poorer and the leaves were lighter green in colour when carbon dioxide was absent from the aeration gas. The differences between the two groups of plants were still very clear on March 16th when the experiment was terminated and photographic records were made. (Plate 1A and B, Plate 2). By this time, however, the nodules of the plants grown in the absence of carbon dioxide in the aeration gas had developed considerably so that the average nodule weight was greater than that of normal nodules (cf Table 1).

When a suspension of killed Rhizobium cells had been added to the culture solution having a reaction of pH 4.8 and from which carbon dioxide was absent the plants had a much better appearance than those grown under similar conditions but in the absence of killed Rhizobium cells. The nodules were, however, concentrated on the main roots in the same way.

Differences in the development of the clover plants due to the presence of CO_2 in the culture solution were less pronounced at a reaction of pH 6.4 than at one of pH 4.8. The differences in distribution of the nodules were, however, similar to those occurring in the two treatments at pH 4.8. Furthermore the colour of the leaves of the plants growing in the culture solution aerated with CO_2-containing air was darker green. This indicated a better nitrogen supply than when carbon dioxide was absent.

Of the plants from all the treatments those having the best appearance were those grown in culture solution having a reaction of pH 6.4 and to which killed Rhizobium cells had been added and which were aerated with CO_2-free air. In this case the nodules were concentrated on the main roots.

The results of this experiment are recorded in Table 1. It will be seen that at a reaction of pH 4.8 the presence of carbon dioxide in the culture solution has increased the yield of the tops by approximately 80 per cent; the yield of the roots was not higher. The relative nitrogen-fixing capacities of the nodules in the presence and absence of CO_2 in the culture solution are indicated by the nitrogen contents of the plant tissues under the two conditions.

PLATE 1. Effect of carbon dioxide in the culture solution on nitrogen fixation and growth of red clover

Series **A** and **B** first experiment (pH = 4.8); CO_2 absent in series **A**, CO_2 present in series **B**.

Series **C** and **D** second experiment (pH 4.7). CO_2 absent from the two pots on the *left* in each series, CO_2 present in the two pots on the *right* in each series.

Series **A**, **B**, and **C** *inoculated* with *Rhizobium trifolii*.

Series **D** *uninoculated* but supplied with potassium nitrate

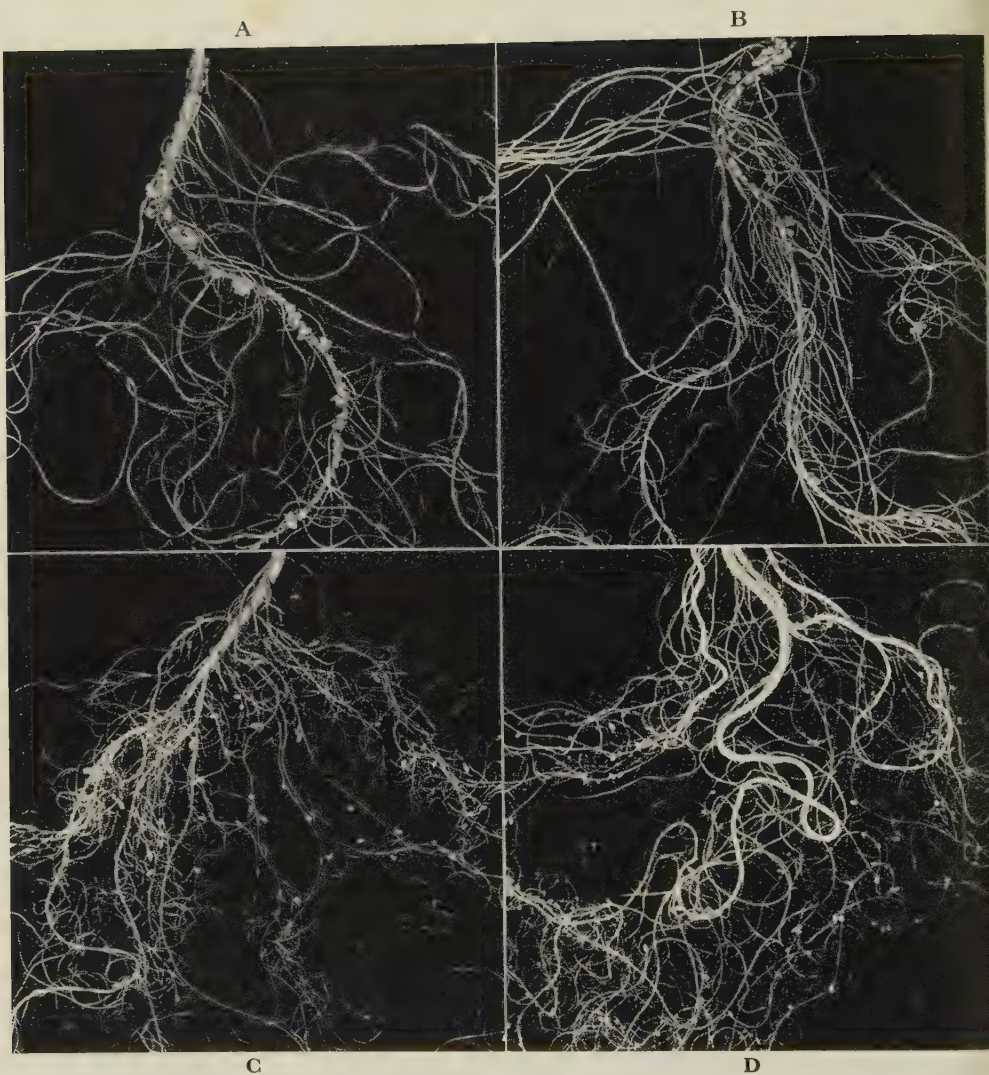

PLATE 2. Effect of carbon dioxide in the culture solution on nodulation of red clover a different pH values. **A** and **B** CO_2 absent, **C** and **D** CO_2 present. **A** and **C** pH 4.8, **B** an **D** pH 6.4.

For the tops the nitrogen contents were 10 per cent greater when the culture solution was aerated with air containing CO_2 and 30 per cent greater in the case of the roots. The total amount of

TABLE 1

Effect of the presence of carbon dioxide and killed Rhizobium cells in the culture solutions, having reactions of pH 4.8 and 6.4, on nodulation, nitrogen content and yield of red clover

Treatment	Tops		Roots + nodules		Nodules		
	Dry matter, g per pot	Nitrogen, % of dry matter	Dry matter, g per pot	Nitrogen, % of dry matter	Number per plant	Average dry weight, mg per pot	Nitrogen, % of dry matter
pH 4.8, no CO_2 . . .	1.08±0.08	2.92±0.12	0.46±0.04	2.31±0.12	126± 27	44±7	7.35±0.35
pH 4.8 + CO_2 . . .	1.79±0.18	3.26±0.07	0.43±0.04	3.01±0.15	214± 37	38±5	8.27±0.19
pH 6.4, no CO_2 . . .	1.69±0.22	3.00±0.08	0.53±0.04	2.25±0.18	217± 40	34±9	7.55±0.27
pH 6.4 + CO_2 . . .	2.00±0.13	3.25±0.07	0.45±0.04	2.74±0.17	446±115	40±6	7.31±0.18
pH 4.8, no CO_2 + killed Rhiz. cells . .	1.70±0.13	3.08±0.14	0.62±0.04	2.61±0.15	284± 24	52±8	7.32±0.41
pH 6.4, no CO_2 + killed Rhiz. cells . .	2.79±0.34	3.01±0.12	0.72±0.09	2.75±0.14	350± 30	79±9	7.19±0.50

nitrogen fixed per plant was approximately 150 per cent greater in the case of CO_2 treatment of the culture solution. In two similar series in which the reaction of the culture solution was pH 6.4 the differences in yield of plant tissue and nitrogen content of plant tissue were smaller than when the reaction was pH 4.8 but they were none the less clearly perceptible. The use of killed suspensions of Rhizobium cells instead of carbon dioxide gave similar results at both pH values.

b. *Investigation into the influence of carbon dioxide and combined nitrogen in the culture solution on the growth of red clover*

A second experiment on the influence of carbon dioxide in the culture solution on the growth of red clover was started on May 15th, 1959. The reaction of the culture medium was maintained between pH 4.6 and pH 4.8. Plants provided with combined nitrogen received 100 mg N as KNO_3 per l. Glass jars of 2-l capacity and containing one plant per jar were employed.

The experiment consisted of the following six series, each series consisting of ten plants in ten containers. *1*) Aeration of the nutrient solution with air freed from carbon dioxide. *2*) Aeration during the second half of the experimental period only with air containing approximately 4% carbon dioxide; during the first half of this period the nutrient medium was aerated with air freed from carbon dioxide. *3*) Carbon dioxide supplied to the culture solution during the

first half of the experimental period only. *4)* Carbon dioxide supplied to the culture solution during the entire experimental period. The plants of Series *1*, *2*, *3*, and *4* were inoculated on May 25th with an effective strain of *Rhizobium trifolii*. Series *5* and *6* were not inoculated with Rhizobium. The plants of these two series were provided with nitrate nitrogen. Series *5* was aerated with air free from carbon dioxide and Series *6* with air containing approximately 4% carbon dioxide.

The results of this experiment were in general similar to those of the previous one. On June 20th the leaves of the plants which had grown in the absence of CO_2 in the culture solution were somewhat lighter green in colour than those of plants grown with carbon dioxide in the culture solution. The roots of the former were longer, thicker and less branched; they had fewer nodules and these were larger in size and white in colour. On this date the CO_2 supply to Series *2* commenced and that to Series *3* ceased.

On July 6th, when the experiment was terminated, the plants which had grown in culture solution aerated with air free from carbon dioxide throughout the experimental period showed the poorest development (Plate 1C; Table 2). Plants grown in culture solution aerated with carbon dioxide-containing air during the

TABLE 2

Effect of the presence of carbon dioxide in the culture solution * on nodulation, nitrogen content and yield of red clover plants when inoculated and when uninoculated, but supplied with potassium nitrate

Series no.	Treatment	Tops		Roots		Nodules		
		Dry matter, g per pot	Nitrogen, % of dry matter	Dry matter, g per pot	Nitrogen, % of dry matter	Number per plant	Average dry weight, mg per pot	Nitrogen, % of dry matter
	Inoculated							
1	No CO_2	0.82±0.09	3.37±0.13	0.21±0.02	2.47±0.09	201±52	41 **	6.99±0.20
2	CO_2 during 2nd half of growing period .	0.86±0.09	3.49±0.09	0.21±0.02	2.58±0.21	225±31	56	6.75±0.37
3	CO_2 during 1st half of growing period .	1.13±0.12	3.58±0.07	0.20±0.02	2.94±0.26	308±40	40	7.43±0.06
4	+ CO_2	1.13±0.14	3.51±0.11	0.22±0.02	2.69±0.12	340±48	40	7.21±0.14
	Uninoculated, + KNO_3							
5	No CO_2	1.43±0.24	4.08±0.10	0.33±0.04	3.44±0.09	—	—	—
6	+ CO_2.	1.54±0.25	4.06±0.07	0.35±0.03	3.61±0.01	—	—	—

* pH = 4.7.
** Calculated from fresh weight and average dry-matter content of the nodules.

second half of the experimental period showed a somewhat better growth and a somewhat darker green leaf colour which, however, was not reflected in the yield of plant tissue nor in its nitrogen content. Omission of the supply of carbon dioxide to the culture solution during the second part of the experimental period gave

plants similar to those grown in a culture solution supplied with CO_2 throughout the experimental period. In both cases the leaves had a darker green colour and the yields were higher than those of the control plants.

The effect of the presence of carbon dioxide on the root system and on nodulation was similar to that observed in the previous experiment *viz* the development of a well-branched root system with thin roots and many pink nodules which were distributed throughout the root system. In the absence of carbon dioxide the roots were thicker and less branched. The nodules were, in general, concentrated along the main roots and the pink colour was not so pronounced. Nodulation in plants receiving CO_2 during the first part of their growing period resembled that of normal plants. The absence of CO_2 during this period resulted in a nodulation condition similar to that of the plants deprived of carbon dioxide over the whole of the experimental period. Apparently the presence of carbon dioxide in the nutrient medium is more important during the first part of the growing period than during the second.

Treatment with potassium nitrate resulted in larger plants whose leaves were also darker green in colour. In this case there was practically no effect of carbon dioxide on the appearance of the tops. (Plate 1D). The roots were somewhat more branched and slightly thinner than in the absence of carbon dioxide.

Nodules of plants from this experiment which had been grown in the absence and presence of carbon dioxide were analysed for amino acids and organic acids by the paper-chromatographic methods described above. Since the amount of nodule tissue available was limited, only one sample from each treatment was analysed. Nodules from plants which had been provided with adequate supplies of carbon dioxide throughout the experimental period were found to contain slightly higher amounts of amino acids and considerably higher amounts of malonic, malic, and α-ketoglutaric acids than those from plants deficient in carbon dioxide.

In a third experiment with red clover, carried out in the spring of 1960, the effect of the presence of carbon dioxide in the culture medium was found to be less pronounced than that observed in the two preceding experiments. The culture solution used on this occasion had the same composition as before except that iron was

supplied either as $FeCl_3$, or as EDTA-complex. In the absence of CO_2 from the culture medium the plants grew less vigorously and their leaves had a lighter green colour than was the case with plants receiving CO_2. The differences in the root systems and nodulation observed in the earlier experiments did not occur, however.

c. *Experiments with peas* (*Pisum sativum*) *and beans* (*Phaseolus vulgaris*).

The nutrient solution used in this experiment had the same composition as described above, except that iron was supplied as $FeCl_3$ (5 mg Fe per l) and nitrogen was given in somewhat lower amounts ($7^1/_2$ mg NH_4NO_3 per l to the pea plants, and $7^1/_2$ mg NH_4NO_3 and 35 mg KNO_3 per l to the bean plants). When carbon dioxide was not supplied to the nutrient medium the *pea* plants showed pronounced nitrogen-deficiency symptoms. Their leaves were yellow-green in colour and many of the lower ones died at an early stage. Plants growing in culture solution aerated with 3 to 5% CO_2 had dark-green foliage and there was no dying-off of the lower leaves (Plate 3A). The number of nodules (approximately 500 per plant in both cases) was unaffected by the presence of carbon dioxide in the nutrient solution, but the fresh weight of the nodules from plants which had received CO_2 treatment was about 20 per cent greater. The colour of the nodules of plants grown in the absence of CO_2 in the culture solution was yellow or greenish compared with the pink colour of the nodules of plants supplied with CO_2. Nitrogen fixation by the yellow or greenish nodules was poor (see Table 3).

In the case of *bean* plants the effect of the presence of carbon dioxide in the nutrient solution was already visible before root nodules had developed. Plants growing in the absence of CO_2 in the nutrient medium were somewhat smaller and their root systems were slightly less branched. The latter formed only a few nodules in contrast to the much more numerous nodules formed by the plants supplied with CO_2. In both cases the nodules were present in the upper parts of the root system (Plates 4A and B) and in both cases the appearance of the nodules was the same. As a result of poor nodulation the plants grown in the absence of CO_2 in the nutrient medium were deficient in nitrogen. The leaves of these plants were yellow green in colour in contrast to the dark green of the leaves of plants treated with CO_2. Resulting from the differ-

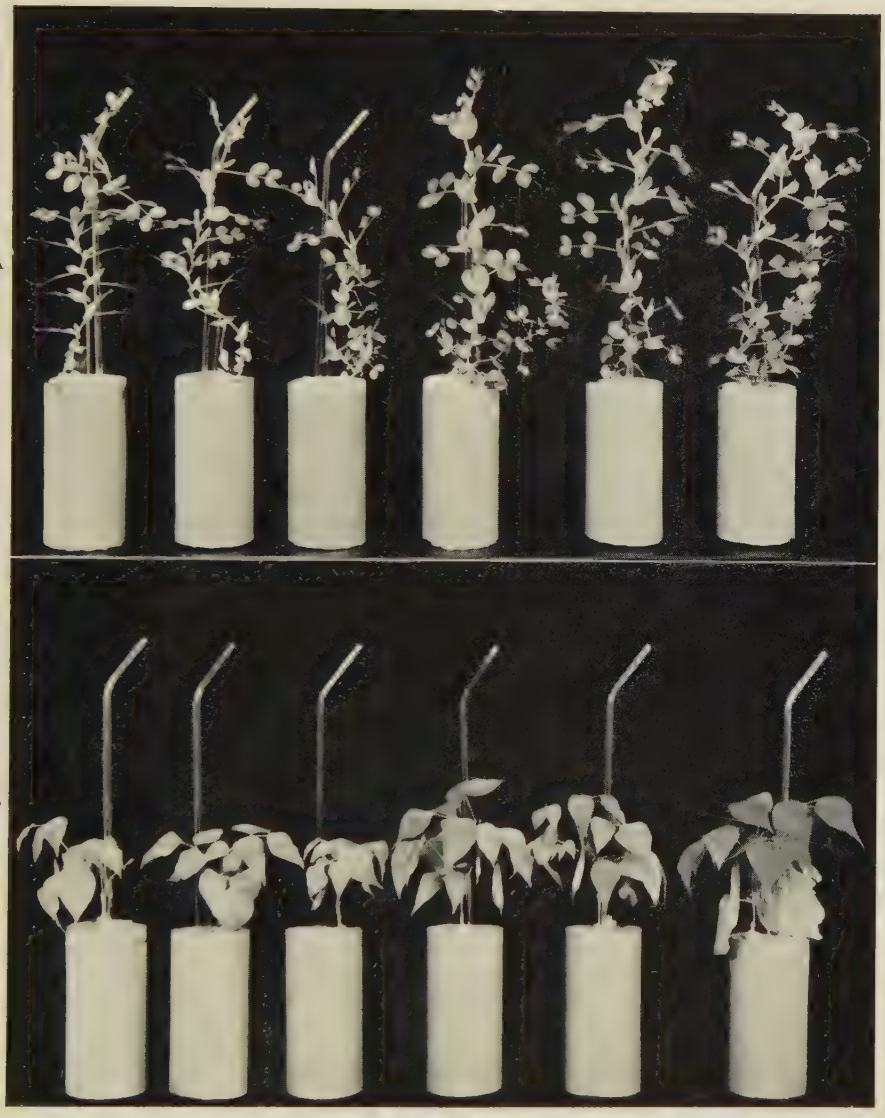

PLATE 3. Effect of carbon dioxide in the culture solution on nitrogen fixation and growth of **A**: pea, and **B**: bean plants at pH 4.8. In each series the three plants on the *left* had grown in the absence of CO_2 in the culture solution, the three on the *right* had grown in culture solution supplied with CO_2.

PLATE 4. Upper parts of the root systems of bean plants grown in nutrient media having a reaction of pH 4.8. **A**: aerated with air free from carbon dioxide. **B**: aerated with air containing 4% CO_2.

TABLE 3

Effect of carbon dioxide in the culture solution on nitrogen fixation and yield (g dry weight per plant) of inoculated pea (*Pisum sativum*) and bean plants (*Phaseolus vulgaris*), grown at pH 4.8. (In parentheses: — the standard deviation of the mean)

Plant	Treatment	Tops		Pods		Roots		Nodules *		
		Yield	N % ‡	Yield	N %	Yield	N %	Total	Primary	Secondary
Pea	CO_2 absent	1.32 **	3.18	—	—	0.40	2.52	1.40	—	—
		(0.13)	(0.09)			(0.05)	(0.06)	(0.14)		
	CO_2 supplied	2.20 **	3.48	—	—	0.45	2.81	1.74	—	—
		(0.22)	(0.07)			(0.05)	(0.05)	(0.29)		
Bean	CO_2 absent	1.02 †	1.74	0.40	2.52	0.60	1.97	1.33	0.28	1.05
		(0.07)	(0.15)	(0.02)	(0.10)	(0.02)	(0.06)	(0.41)	(0.11)	(0.37)
	CO_2 supplied	1.73 †	2.18	1.01	2.67	0.62	1.89	2.84	2.32	0.53
		(0.20)	(0.37)	(0.28)	(0.18)	(0.02)	(0.11)	(0.37)	(0.47)	(0.42)

* Fresh weight, g.
** Including pods.
† Excluding pods.
‡ Per cent of dry matter.

ences in nitrogen fixation, the plants grown in the presence of CO_2 in the nutrient medium gave considerably higher yields (Table 3, Plate 3B). At a later stage of development the plants grown in the absence of CO_2 in the culture solution formed many small secondary nodules in the more distal parts of the root systems. Plants grown in culture solution supplied with CO_2 formed fewer secondary nodules, presumably as a result of the presence of effective primary nodules. No explanation of the formation of the secondary nodules in plants growing in the absence of CO_2 in the nutrient medium can be given.

d. *Effect of carbon dioxide treatment of the soil on the growth of red-clover plants in acid soil.*

Three kinds of experiment have been carried out to study the effect of CO_2 treatment of acid soil on nodulation and growth of red clover. The first type consisted of aeration of the soil with either air or a nitrogen—oxygen mixture enriched with 5% carbon dioxide. In some experiments aeration took place continuously, in others intermittently. In the second type of experiment the acid soil, contained in a pot, was divided into two parts.

The soil of the upper half of the pot, in which the plant roots were growing, consisted of untreated acid soil whilst the lower half of the pot contained the same acid soil to which calcium carbonate had been added so that

carbon dioxide might be produced. In some cases a layer of stable manure was added to the soil in the lower half to increase the CO_2 content of the soil atmosphere. The soil in the two halves of the pot was separated by a perforated glass plate covered with a layer of asbestos fibre treated with sulphuric acid to prevent penetration of the roots into the lower half and to prevent the transfer of ammonia to the acid soil of the upper half. A thick layer of glass-wool was present between the layer of asbestos and the soil.

Experiments of the third type were influenced by the following consideration. When the carbon dioxide content of the soil air is augmented by the addition of calcium carbonate to an acid soil it must be assumed that this effect is more pronounced when the carbonate is added at the time of sowing than when the addition is made a number of weeks earlier.

In the three experiments in which the soil was aerated with carbon dioxide-containing air, one resulted in a pronounced response to the treatment (improved nodulation and nitrogen fixation; yield of tops 2.39 g per pot as compared with 1.2 g per pot for the control). A gas mixture consisting of 5% CO_2, 1% O_2, and 95% N_2 was used for aerating the acid soil twice a day for 15 minutes; the control pots were not aerated. In a second experiment in which the aeration was continuous, a slight response to the carbon dioxide treatment was observed (yields of tops from plants from untreated control pots — 0.2 g, from pots aerated with a CO_2-containing gas mixture — 0.6 g, from pots aerated with air — 0.8 g, and from pots aerated with air free from carbon dioxide — 0.3 g. In a third experiment in which acid soil was continuously aerated with air free from CO_2 or with air containing 5% CO_2 the plants showed no response to the presence of the carbon dioxide.

An experiment of the second type gave a slight but clearly perceptible response, with regard to nodulation and nitrogen fixation by the clover, to addition of $CaCO_3$ or stable manure to the acid soil in the lower half of the pot. The respective yields of plant tops were: from control pots — 1.0 g, lower soil treated with $CaCO_3$ — 2.4 g, and lower soil treated with stable manure — 1.7 g.

Two experiments of the third type gave completely divergent results. In both cases a comparison was made between the effect of addition of calcium carbonate to acid soil at the time of sowing of the clover seeds and the effect of additions made two or four weeks before sowing. In the first experiment, $CaCO_3$ added at the

time of sowing resulted in pronounced stimulation of nodulation and considerably higher yields; when supplied two weeks before sowing the effect of added $CaCO_3$ was moderate and at four weeks before sowing nodulation and yield were scarcely greater than in the acid control soil.

In the second experiment the effect of added calcium carbonate on nodulation and yield of the clover was independent of the time of application.

The results of these experiments show that under certain conditions treatment of acid soils with carbon dioxide may have a beneficial effect on nodulation and growth of red clover. In a number of instances, however, the effect was small or absent. Therefore more carefully controlled experiments will have to be carried out, if we are to learn the conditions under which carbon dioxide may promote the nodulation of plants growing in soils.

DISCUSSION

The experiments with culture solutions aerated with air free from carbon dioxide and with air containing approximately 4% carbon dioxide have clearly demonstrated the important effect of this component on nodulation and on nitrogen fixation. In the absence of carbon dioxide from the culture medium the nodules on the roots of red clover were fewer in number and they occurred chiefly on the main roots. When the culture medium was aerated with CO_2-containing air the nodules were distributed throughout the root system. The total weight of the nodules from the red-clover plants grown in the absence of CO_2 in the culture medium was not lower than that of normal plants but their nitrogen-fixing capacity was apparently reduced.

The fact that the roots of the plants grown in the absence of CO_2 in the culture medium were found to be less branched than those of plants growing in culture solution supplied with carbon dioxide suggests that it is the root tissue rather than the bacteria which is affected. This conclusion would be in agreement with the results of some recent investigations concerning the effect of carbon dioxide on plant roots.

Dale [4] found that root-hair development in Anacharis (Elodea) depends on the presence of a relatively high concentration of

carbon dioxide in the nutrient medium. Bergmann [3] obtained evidence that the formation of root hairs in rye seedlings requires the presence of relatively large amounts of carbon dioxide in the culture solution. In experiments with extracts from roots of *Phaseolus vulgaris* Jackson and Coleman [7] observed fixation of carbon dioxide and formation of malic acid arising from the activity of phosphoenolpyruvate carboxylase. Van Die [5], working with intact tomato plants, also observed a rapid fixation of carbon dioxide by the roots, malic acid being the main reaction product.

The finding that nitrogen fixation by clover plants reached higher values when carbon dioxide was present in the nutrient medium than when it was absent could be attributed to the more liberal supply of α-keto acids which, in a preliminary chromatographic test, was found to occur in the nodules. Plants growing in the absence of carbon dioxide in the nutrient medium but provided with a suspension of sterilized Rhizobium cells developed considerably greater numbers of nodules and fixed more nitrogen than the control plants. This may indicate that some component of the Rhizobium extract can function in a similar manner to carbon dioxide.

That the better appearance and the greater production of dry matter in the plants grown in culture solution supplied with CO_2 were due to increased nitrogen fixation may also be concluded from the fact that plants provided with combined nitrogen did not respond to treatment of the culture solution with CO_2.

The results of the experiments with pea and bean plants provide additional evidence that the presence of carbon dioxide in the nutrient medium is essential for symbiotic nitrogen fixation. In the case of pea plants the number of nodules formed was not affected by the CO_2 content of the nutrient solution. In the absence of CO_2 the weight of the nodules was slightly reduced and their colour was yellow or greenish instead of pink as in the presence of CO_2. The nitrogen-fixing capacity of the plants grown in the absence of CO_2 in the culture medium was very poor and in consequence the plants showed pronounced symptoms of nitrogen deficiency.

The number of nodules formed on the roots of bean plants was much reduced when carbon dioxide was absent from the nutrient solution. This resulted in poor nitrogen fixation and nitrogen deficiency of the plants. It could not be decided whether the effective-

ness of the nodules of such plants was also smaller. The secondary formation of nodules which was observed a number of weeks after the formation of the primary nodules, particularly in the nitrogen-deficient plants grown in the absence of carbon dioxide in the culture solution, was presumably due to some unknown secondary factor.

The experiments in which acid soils have been treated with carbon dioxide have not given consistent results. In a number of experiments evidence was obtained that this treatment promoted nodulation and nitrogen fixation in red clover. In further experiments, however, negative results were obtained. No explanation can be given of these divergent results. The results obtained so far do not allow any conclusion to be reached concerning the effect upon nodulation and nitrogen fixation of the increased carbon-dioxide supply resulting from treatment of acid soils with calcium carbonate.

SUMMARY

1. The presence of carbon dioxide in the culture solution was found to be essential for optimal nodulation and nitrogen fixation by red clover plants. In the absence of this compound fewer root nodules were formed and these were mainly concentrated along the main roots whereas in the case of plants provided with carbon dioxide in the nutrient medium, the nodules were distributed throughout the root system.

Nodules of clover plants grown without carbon dioxide in the culture solution fixed lower amounts of nitrogen than those of plants grown in culture solutions containing carbon dioxide. It is suggested that this may have been due to a more liberal supply of α-keto acids which, in a preliminary experiment, was found to occur in the nodules of plants grown in culture solution supplied with CO_2.

The effect of carbon dioxide on nitrogen fixation was more pronounced at a reaction of pH 4.8 than at a reaction of pH 6.4.

When provided with nitrate nitrogen clover plants did not respond to the presence of carbon dioxide in the culture solution.

2. Pea and bean plants growing in a culture solution having a reaction of pH 4.8 responded strongly to the presence of CO_2 in the nutrient medium. The number of nodules formed by the pea plants was not affected when CO_2 was supplied to the culture solution but fixation of nitrogen by the nodules was considerably improved. The number of nodules formed by the bean plants was much lower when CO_2 was absent from the culture medium. Both nitrogen fixation and growth were considerably reduced in these plants when grown in a CO_2-free culture solution.

3. Treatment of an acid soil with carbon dioxide was found to promote nodulation and nitrogen fixation in red clover in some experiments; in other experiments this treatment did not produce any effect.

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THE PRODUCTION OF RYEGRASS LABELLED WITH CARBON-14

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INTRODUCTION

Plant material labelled with carbon-14 is being used in this laboratory to study the turnover of organic matter in soil. The resistant parts of plants, cellulose, lignin, *etc.*, are thought to be important in the formation of "humus"; if this is true then plant material labelled by exposing growing plants to highly active carbon dioxide for short periods is not suitable for our work, as in such material the cellulose and lignin is very lightly labelled, most of the activity being in the water-soluble part of the plant. To obtain plants in which the specific activity of the cellulose and lignin is comparable to that of the water-soluble part, the plants must be grown for long periods in the presence of uniformly labelled carbon dioxide. Several growth chambers have been developed for producing this type of labelled material [1 4 5] but the only one described in detail is the industrial-scaled apparatus of Scully *et al.*[5] at the Argonne National Laboratories of the U.S.A.E.C. We required a growth chamber which could produce up to 100 g dry plant material at any season of the year, quickly, in complete safety, and as cheaply as possible. Absolutely uniform labelling was not required for the work planned but it was desired that the specific activity of any fraction of the plants be within 10 and 15 per cent of the specific activity of the whole plant. This paper describes the construction and operation of a growth chamber meeting these specifications.

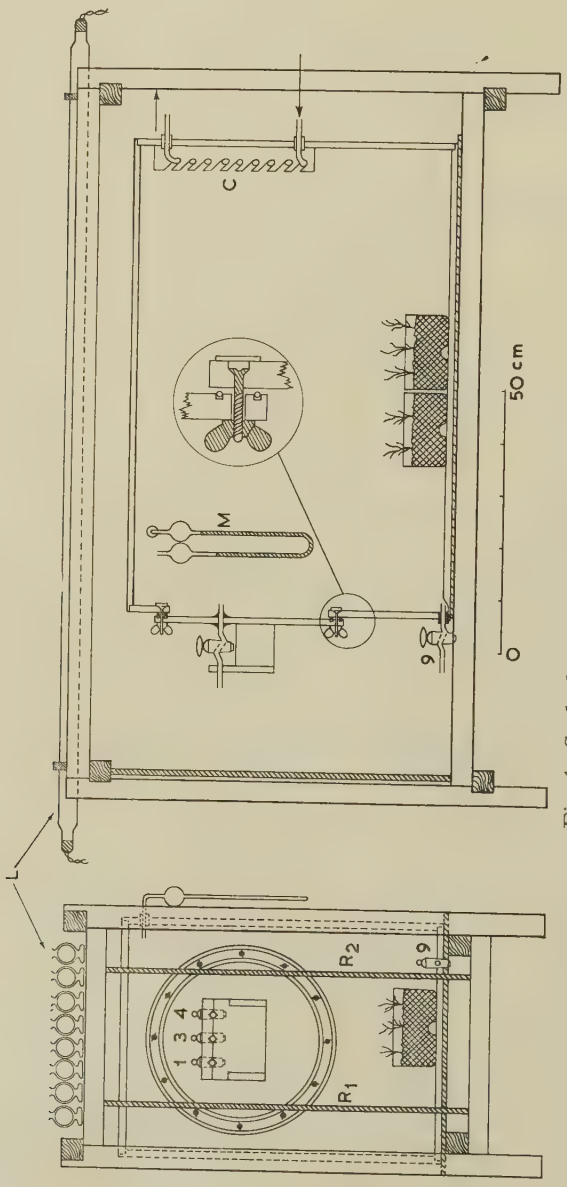

Fig. 1. Scale drawing of growth chamber.

CONSTRUCTION OF THE GROWTH CHAMBER AND ASSOCIATED APPARATUS

Fig. 1 is a scale drawing of the growth chamber, supporting cradle, and lighting system. The chamber, a box roughly 46 × 61 × 91 cm in dimensions, was made from 1.27 cm clear methacrylate ("Perspex") sheet. The chamber, cradle, and all associated apparatus were built inside a fume cupboard. When the chamber was in operation the fume cupboard fan was run continuously, partly for additional cooling, and partly to insure that no radioactive gas entered the laboratory in case of an accident to the chamber. The glassware in the gas-handling system, shown diagramatically in Fig. 2, was carried by

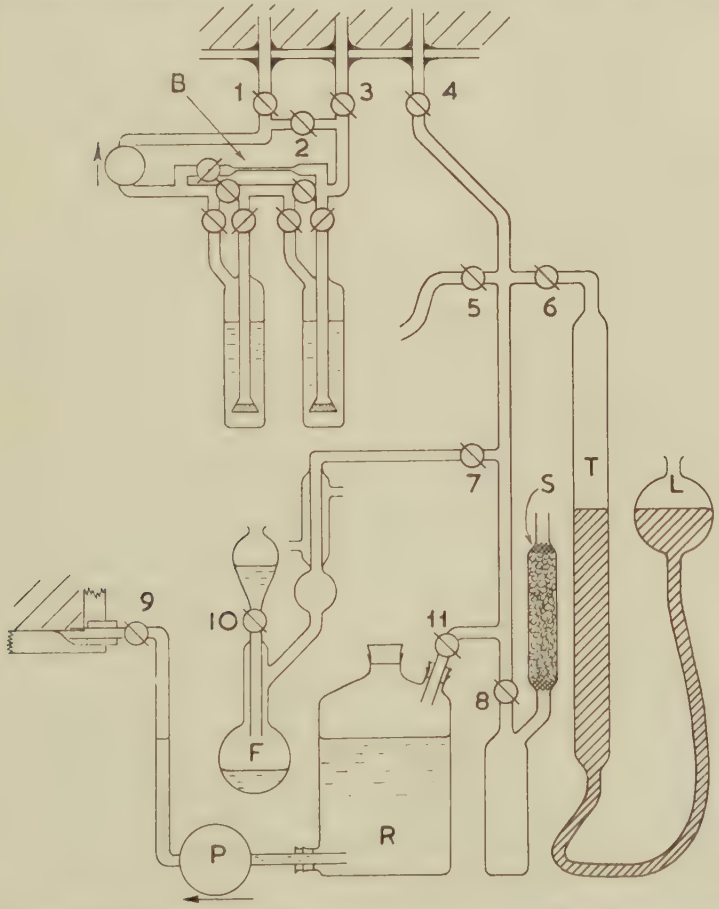

Fig. 2. Schematic diagram of apparatus for circulating gas, pumping and storing nutrient solution, generating CO_2, and adjusting pressure in the chamber.

two rods (R_1 and R_2, Fig. 1) mounted on the end of the cradle. All taps in the assembly were of high-vacuum, oblique, spring-loaded type. The ground glass joints were spring loaded and when flexible connections were needed clear polyvinyl chloride tubing, wired and waxed at the joints, was used. The whole apparatus held a vacuum of 6 cm of mercury for 24 hours with no detectable leak, but there was a slight loss of carbon dioxide: 1.0 g of carbon dioxide was placed in the chamber and 28 days later 8.2 per cent of this had been lost, mainly by diffusion through the rubber ◯-rings on the door of the chamber.

Gas circulation

A diaphragm pump (Compton Type D, Series 2) circulated gas through two dreschel bottles, the first containing 20% potassium hydroxide and the second water. A bleed tube (*B*, Fig. 2) was connected across the dreschel bottles, partly bypassing them and thus making control of the system less critical. This part of the apparatus was only used to remove unlabelled atmospheric carbon dioxide from the chamber at the beginning of a run and unused labelled carbon dioxide at the end of a run.

Pressure control

The chamber was designed to run slightly below atmospheric pressure to minimise the escape of radioactive gas should a leak occur. During a run the pressure in the chamber (indicated by the liquid-paraffin manometer *M*, Fig. 1) gradually increased because the volume of gas removed by daily sampling was less than the volume of oxygen produced by photosynthesis, particularly in the later stages of a run when large volumes of carbon dioxide were being added frequently. The pressure was adjusted by a simple pumping system consisting of a mercury manometer (*T*, Fig. 2) and levelling bulb (*L*, Fig. 2), excess gas being forced out through the soda lime tube (*S*, Fig. 2) after taps *4*, *5*, *7*, and *11* had been closed, and taps *8* and *6* opened. The pressure difference was kept as near as possible to 15 cm of liquid paraffin (S. G. 0.870) during a run. If the pressure difference was greater than this, air was admitted through the soda lime tube (*S*, Fig. 2) by opening taps Nos. *8* and *4*.

Carbon dioxide generation

Carbon dioxide was generated in flask *F* (Fig. 2) by adding excess of 80% lactic acid to a measured volume of sodium carbonate solution at the required specific activity, the gas being admitted to the chamber via taps *7* and *4*. After frothing had subsided the contents of the flask were boiled under reflux for two minutes, tap *7* then closed and the carbon dioxide in the gas lines mixed with the chamber gas by raising and lowering the mercury in *T*, Fig. 2.

Determination of carbon dioxide in the chamber gas

A sample of the chamber gas was collected in an evacuated flask of known volume (1168 ml) via tap *5*, Fig. 2, an excess of 0.015*N* barium hydroxide

solution containing 0.2*N* barium chloride and 0.1% capryl alcohol added, the flask shaken vigorously for 30 minutes, and the unused barium hydroxide titrated against standard 0.01*N* hydrochloric acid containing 0.2*N* barium chloride, phenolphthalein being used as indicator.

Illumination

Eight five-foot fluorescent lamps (Philips T.L.F. 80 w. Warm White) were mounted above the chamber (*L*, Fig. 1). The sides of the chamber were covered with bright aluminium foil and with this foil in position the light intensity on the floor of the chamber was 1750 foot-candles, rising to 2550 foot-candles at the top, the relationship between distance from the lights and light intensity being roughly linear inside the chamber. The variation in light intensity across the floor of the chamber was less than 5 per cent.

Temperature and humidity control

Neither the temperature nor the humidity were controlled to fine limits: the temperature variations inside the chamber during a typical run can be seen from the lower part of Fig. 3. Heat from the lighting system was removed by a water-cooled tin coil (surface area 0.3 sq. m) mounted inside the chamber (*C*, Fig. 1). Water transpired by the plants condensed on the cooling coil and ran back into the nutrient reservoir.

Plant support

The plants were grown in shallow polystyrene trays 14 cm square filled to a depth of 5 cm with washed crushed quartz (3.5 to 2.0 mm mesh). Holes pierced in the bottom of the trays were covered with loosely fitting convex perspex plates (see Fig. 1) to allow free entry and exit of nutrient solution. It was most important to maintain free drainage as plants soon died in water-logged trays.

Nutrient solution

The standard complete nutrient solution recommended by Hewitt [2] was used at either the higher or lower of the two suggested nitrogen levels, the level depending on the number of plants in the chamber. The solution was stored in the dark in a reservoir (*R*, Fig. 2) below the level of the growth-chamber floor, and could be pumped into the chamber by a pump (*P*, Fig. 2) controlled by a time switch. The growth chamber was set at a slight angle so that the nutrient solution drained back into the reservoir when the pump switched off. The floor of the chamber was coated with a silicone grease which aided drainage by preventing wetting by the nutrient solution. It was essential that the nutrient solution did not cover the surface of the quartz in the trays, *i.e.* the chamber was not flooded to a depth greater than 4 cm. These precautions confined algae to an area immediately around the bottom of the trays containing the plants, but if they were not observed both the surface of the quartz in the trays and the whole chamber floor rapidly became covered by a film of algae.

RESULTS

Operation of the growth chamber during a typical run

Preliminary runs with non-labelled carbon dioxide showed that, of the various ryegrass strains examined, Dutch Westerwolth was the most tolerant of conditions in the growth chamber. This strain grew rapidly and flowered under continuous illumination. The seeds were sown in trays (4 trays, 100 seeds per tray) containing crushed quartz, germinated at 25°C in the dark, and the trays placed in the growth chamber when the first leaf showed above the surface. The chamber was closed, the gas-circulating system run until the carbon dioxide concentration was about 0.01% by volume, a measured charge of labelled carbon dioxide admitted (generated from a sodium carbonate solution containing 0.009 g carbon/ml at an activity of 32.8 μc/g carbon), the lights switched on and run continuously until harvest. Five litres of nutrient solution at the lower of the two nitrogen levels recommended by Hewitt [2] was placed in the reservoir (*R*, Fig. 2) and pumped into the chamber once a day for 15 minutes. During the run the chamber gas was sampled daily and the carbon dioxide content determined. The uptake of carbon dioxide was plotted as the experiment proceeded and a fresh charge of labelled carbon dioxide added when it was calculated from the graph that the level had fallen to about 0.1% by volume. Fig. 3 shows the carbon uptake during run B, calculated from the amount already added to the chamber (stepped graph) by deduction of that present as carbon dioxide at a given time. Although the carbon dioxide levels, rising on occasion to 1.5% by volume, were much above normal atmospheric the plants did not appear abnormal. It is possible, however, that the slow growth during the first 12 days of illumination (Fig. 3), may have resulted from the high carbon dioxide concentration, and that after this period the plants were acclimatized. No visible sign of radiation damage is to be expected [5] with the specific activity of carbon-14 used (the radiation dosage was approximately 8 rep/day), and none was seen. During the course of the run the temperature on the outside of the growth chamber varied from 8° to 22°C, that inside from 16° to 23°C, and the temperature of the water in the cooling coil from 11° to 12.5°C. Fig. 3 shows that these temperature fluctuations had no detectable effect on the rate of uptake of carbon dioxide by the plants.

At the end of the run the plants were first allowed to reduce the carbon dioxide concentration to their compensation level by photosynthesis and then the gas circulation system was switched on for 16 hours, the lights being kept on. In this way the carbon dioxide

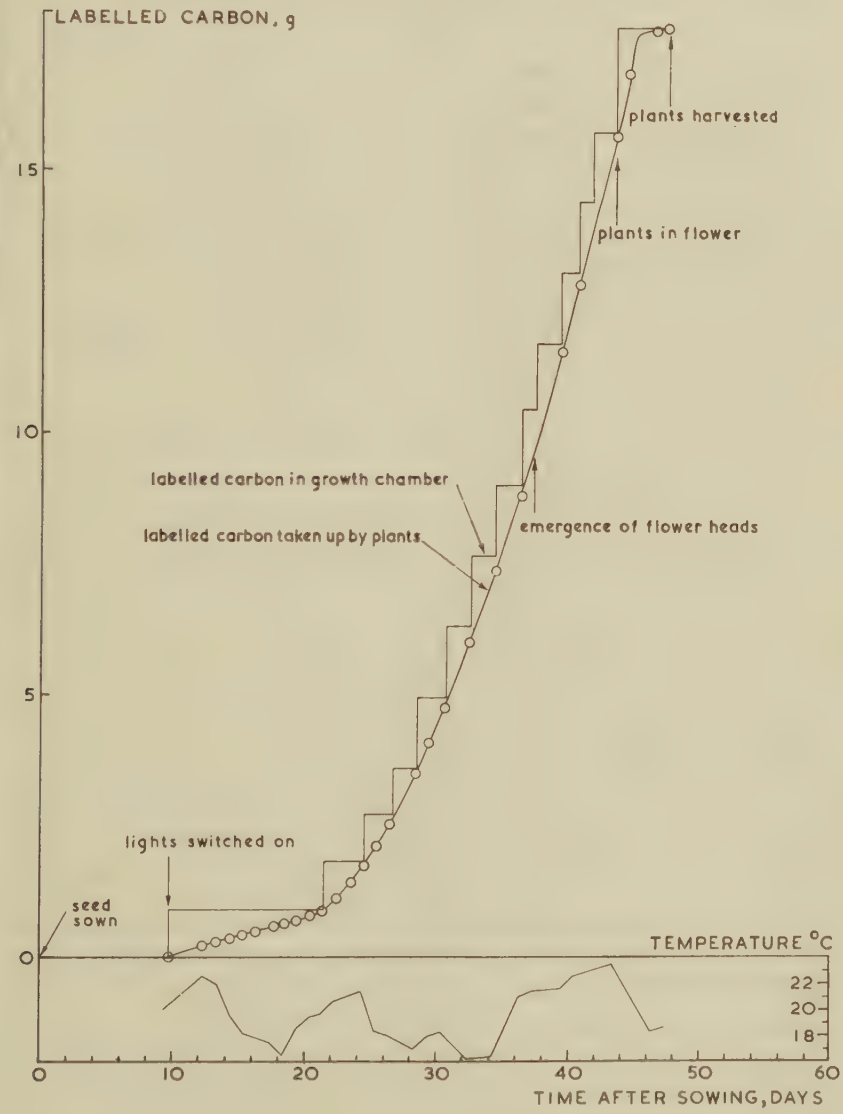

Fig. 3. Uptake of labelled CO_2 by Westerwolth ryegrass. Lower part of figure shows the temperature inside the chamber during the run.

content of the chamber was reduced to 0.01% by volume, with an activity of approximately 0.4μc. The chamber was opened, the trays removed and the aerial parts of the plants cut off as close as possible to the roots. The tops were cut into 2-cm lengths by scissors, transferred to wide necked jars, the jars covered with butter muslin and the plant material freeze-dried in these jars to constant weight. The roots were separated as completely as possible from quartz grains by shaking in water, as much water as possible was squeezed out, and the roots freeze-dried. The dry plant material was ground to pass a 0.5 mm sieve in a Glen Creston micro hammer mill, the mill being mounted in a glove box maintained at a pressure slightly below atmospheric. Great care was taken to avoid swallowing or inhaling the dust produced during grinding, as plant dust which contains insoluble materials like cellulose or lignin is unlikely to be eliminated from the body as rapidly as are soluble carbon compounds [8].

Yield of labelled plant material

To draw up a carbon balance sheet for the growth chamber it was necessary to determine the carbon content and specific activity of the seeds, the labelled sodium carbonate solution, and the plants after harvest. The carbon content of the plant material was determined by Shaw's [6] method in which a sample containing approximately 50 mg carbon was oxidised by acid dichromate and the carbon dioxide collected and weighed in a U-tube containing soda lime. The specific activity of the carbon was found by measuring the activity of the known amount of carbon dioxide absorbed in the U-tube and comparing it with that of a source containing carbon-14 of known activity. For this the U-tube was filled in a slightly different way from that used by Shaw. About 20 g of soda lime (Carbosorb, 10–16 mesh, obtained from B.D.H. Ltd.) was packed in the tube (diameter 15 mm), a cotton-wool plug placed in either side of the soda lime, anhydrous magnesium perchlorate (14–22 mesh) placed in each arm of the U-tube above the soda lime and the tube closed with rubber bungs. After absorption of carbon dioxide the rubber bungs were removed, most of the magnesium perchlorate tipped out, and the soda lime, cotton wool plugs and any residual perchlorate emptied into a 12-oz. wide-necked bottle containing 5 ml of water and 2 drops of capryl alcohol. A 4.00*N* solution of potassium hydroxide (8.0 ml) was placed in a test tube graduated at 10 ml and

the tube placed in the bottle which was then stoppered with a bung carrying a tap. The bottle was evacuated to a pressure of about 10 cm Hg, 80 ml of 30% nitric acid run in through the tap, which was then closed and the bottle allowed to stand for 5 days. The acid completely dissolved the soda lime, liberating the carbon dioxide which was absorbed in the alkali tube. Tests with labelled carbon dioxide showed that recoveries from soda lime by this method were 99.3-100 per cent. On removal the alkali tube was shaken, 1 ml of a 1.6% solution of Napthalene Leather Carbon G.S. in water added, the whole brought up to the 10-ml graduation in the test tube, and the carbon-14 concentration determined by scintillation counting (Jenkinson [3]). A solution containing the same amount of potassium hydroxide and dye was used to obtain the background count. Before and after each series of measurements the standard source (a carbonate solution of known activity) was counted and all activities expressed in terms of this source.

Table 1 gives the carbon balance sheet for labelled and unlabelled carbon in run B. There is an overall loss of 1.61 g of carbon-12 and 57μc of carbon-14, due to the loss of labelled carbon dioxide by diffusion through the rubber and plastic parts of the chamber, to the growth of algae (which were not harvested), and to small portions of root lost when the roots were shaken with water.

TABLE 1

C^{12} and C^{14} balance sheet for ryegrass (Dutch Westerwolth) grown in chamber

Balance sheet	Wt., g	Carbon, %	Wt. carbon, g	Specific activity μc/g carbon	Carbon-14, μc
Material added					
Atmospheric CO_2 in chamber	0.03	—	0.01	0	0
Seeds	1.48	43.0	0.64	0	0
Labelled Na_2CO_3	156.43	—	17.71	32.8	581
Material removed					
Labelled CO_2 in gas samples	0.15	—	0.04	32.8	1
Labelled CO_2 not taken up by plants	0.04	—	0.01	32.8	0.3
Plant tops	32.6	41.8	13.63	31.4	428
Plant roots	8.4	36.5	3.07	31.1	95
Losses	—	—	1.61	—	57

Uniformity of labelling of plant material

The distribution of radioactivity in plant material grown in the growth chamber can be non-uniform for a number of reasons. Diffusion and/or leakage of carbon dioxide through the chamber and associated apparatus can result in loss of carbon-14 and dilution of the chamber gas with atmospheric carbon dioxide. Some unlabelled carbon (with run B, about 4 per cent of that present in the plants at harvest) is present in the seeds. This unlabelled carbon may or may not be distributed uniformly through the plants as they grow. Finally isotopic fractionation might occur during metabolism and this could conceivably lead to a preferential enrichment (or depletion) of some plant constituent in carbon-14. Table 2 shows the result of a fractionation of plants from run A using a much simplified version of the scheme suggested by Waite and Gorrod [7] for

TABLE 2

Distribution of activity in labelled ryegrass (Dutch Westerwolth)

Plant part	% of total dry matter of plant	Specific activity, μc/g carbon	Standard error
Tops			
Unfractionated	100	23.8 (3)*	± 0.21
Part soluble in alcohol/benzene	10.3	—	
,, ,, ,, hot water	35.6	24.6 (2)	± 0.26
,, ,, ,, 17.5% KOH	20.2	23.8 (2)	
Insoluble residue	34.0	22.5 (2)	
Roots			
Unfractionated	100	21.0 (4)	± 0.18
Part soluble in alcohol/benzene	6.8	—	
,, ,, ,, hot water	24.3	23.6 (2)	± 0.26
,, ,, ,, 17.5% KOH	19.5	20.1 (2)	
Insoluble residue	49.4	19.4 (2)	

* Number of determinations in parenthesis

the analysis of grasses. The ground plant material was extracted with ethanol/benzene under reflux for 18 hours, then with water at 60°C for 30 minutes, then with 17.5% potassium hydroxide at room temperature under nitrogen for 30 minutes. The specific activity of the water-soluble part, the alkali-soluble part (containing the hemicelluloses, part of the lignin, and part of the protein), and the residue (mainly cellulose) were determined by the method described above. The results show that the labelling is not uniform,

that the tops have a higher specific activity than the roots and that the water-soluble part has a higher specific activity than the cellulose fraction. In run A (specific activity of labelled carbon dioxide: 25μc g carbon) the plants were placed in the chamber 18 days after germinating so that more unlabelled carbon was present than in run B, where the plants were placed in the chamber directly the first leaf appeared above the surface of the quartz (4 days after germinating). Although the plant material from the later run was not fractionated, Table 1 shows that the difference in specific activity of the roots and tops is not significant, unlike that between roots and tops from run A.

CONCLUSIONS

For work where radiocarbon must be distributed throughout a plant to a high degree of uniformity the seed should be placed in the chamber immediately after germination. For less precise work the plants can be well established before being placed in the chamber. The largest yield from any one run with the chamber was 80 g dry matter (from 12 trays of plants) but this could easily be increased to 100 to 120 g by using the maximum number of trays (18). To produce more than 50 g of plant material, 10 litres of culture solution containing nitrogen at the higher of the two levels recommended by Hewitt [2] should be used.

Automatic monitoring of the carbon dioxide level and carbon-14 activity would be a very convenient, if expensive, aid to running the chamber, but in our experience sampling and chemical analysis was satisfactory for following the carbon dioxide content of the chamber atmosphere.

SUMMARY

The construction and operation of a growth chamber for producing plant material labelled with carbon-14 to a specified degree of uniformity is described.

The specific activity of the plant material is measured by a method based on scintillation counting.

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PRODUITS D'HUMIFICATION DES SCIURES

QUELQUES CARACTERISTIQUES PHYSIQUES ET CHIMIQUES

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Nous avons présenté récemment deux notes [2] [3] consacrées à l'humification de débris ligneux et nous tenterons ici de caractériser, grâce à quelques critères simples, les substances humiques formées. Dans ce but, nous déterminerons la densité optique dans la région visible du spectre, le taux de coagulation par $CaCl_2$ et le rapport C/N.

I. MATÉRIEL ÉTUDIÉ

1°) Nous nous sommes adressés, à titre de référence à des substances extraites de différents sols:

S. Acides humiques d'un mull calcimorphe, riche en matière organique et Ca.

A. Acides humiques de l'horizon A_0 d'un podzol sur grès vosgien.

R. Acides humiques obtenus, sans décalcification préalable, à partir d'une rendzine forestière (Forêt de Haye, Nancy).

Acides fulviques provenant de l'horizon d'accumulation (B_2) du podzol ci-dessus.

2°) Nous avons déterminé la densité optique des acides humiques de quelques échantillons décrits dans nos notes antérieures, savoir:

a) Extraits par NaOH 0,1*N*:
C11, C14, C15, D6 et D8 (cf. [3])

b) Extraits par NaF 1%: n° 2, sciure attaquée, *in vitro*, par un inoculum mixte non lignivore; n° 3, sciure attaquée par une culture pure de *Trechispora* sp. [2]; n° 2 + 3, sciure attaquée successivement par l'un puis l'autre des agents précédents. L'ordre d'attaque n'influe par sur les caractères du produit final.

3°) Nous avons étudié 10 échantillons recueillis à Génicourt-s-Meuse en Janvier et Février 1960 et âgés pour la plupart de 3 – 4 ans. Il s'agit de:

a) produits acides (pH 3,5 à 4,7) à taux de lignine réduit (20–22%)

Q: sciure blanc-jaunâtre, pulvérulente, attaquée par *Clitocybe cyathiformis*.

T: copeaux de même teinte, de structure mieux conservée, à forte odeur de *Trechispora* sp.

U: situé au-dessous de T, à une profondeur de 15 cm environ, présente des caractères analogues, mais sa structure est très altérée.

L: copeaux très dégradés, presque superficiels, gris-brun clair, portant les fructifications de *Bolbitius vitellinus*.

N: produit présentant exactement l'aspect de l'échantillon D6 mais moins âgé et de pH acide (4,1).

b) produits voisins de la neutralité biologique (pH 5,5 à 7,2), à teneur forte en lignine (37 à 40%):

W: Copeaux de structure analogue à celle de U et situés au-dessous de ce dernier sous quelque 40 cm. Brun-tabac, le produit ressemble à première vue aux spécimens C5 et C6, eux aussi jeunes et profonds.

K et X: presque superficiels, peu altérés, brun-noir à noir. Il s'agit de produits comparables aux échantillons C7 et C8. Cependant la région périphérique du tas à laquelle ils appartiennent est déjà envahie de rhizomes.

P: copeaux de teinte sombre comme les précédents mais de structure très altérée et portant *Pluteus cervinus*. Ils semblent analogues à l'échantillon C14.

M: recueilli au point même du prélèvement C12, nous n'y avons jamais observé de stade lignivore depuis 4 ans; le produit a l'aspect d'un terreau grossier d'un noir intense mais nous verrons qu'il est beaucoup moins évolué que D8.

4°) nous avons examiné les acides humiques formés dans des cultures pures de 8 mois de:

n° 20, un Hyménomycète lignivore non identifié (culture sans $CaCO_3$).

n° 5, *Mammaria echinobotryoides* (en présence de $CaCO_3$).

II. TECHNIQUES D'ÉTUDE

A. *Obtention des acides humiques*

Le produit frais, épuisé par l'eau, est extrait par NaOH 0,1*N* ou NaF 1% (2 agitations de 15 minutes séparées par un repos de 14 h.). La liqueur filtrée est précipitée par 2% de HCl 10*N*. La purification est assurée par 3 dissolutions (NaOH 0,1*N*) alternant avec 2 précipitations (HCl 2%), suivie de filtration sur verre fritté et de dialyse de 3 jours contre de l'eau distillée. La solution est enfin titrée par le mélange sulfo-chromique (Méthode Anne [1])

B. *Densite optique*

La solution dialysée est diluée à 136 mg de C par litre à l'aide d'une solution de $NaHCO_3$ 0,02*N* puis examinée aussitôt en cuve de 1 cm.

C. *Coagulation par $CaCl_2$*

La solution dialysée est additionnée de 1/5 vol. de $NaHCO_3$ 0,1*N* puis amenée à 136 mg de C/l par une solution tampon de pH 7,5 (H_3BO_3-KCl-Na_2CO_3). A 5 ml de cette dilution on ajoute 3 ml de solution de $CaCl_2$ de

manière à obtenir des concentrations finales de 20, 30, 40 et 80 me de Ca/l. Après 24 h les mélanges sont centrifugés [4] et la densité optique du surnageant mesurée par rapport au tampon. La valeur obtenue est alors calculée en % de la densité optique d'un témoin sans Ca; enfin le taux de coagulation est exprimé en soustrayant ce dernier chiffre de 100. Le résultat est indépendant de la longueur d'onde choisie, mais nous avons toujours opéré à 450 mμ, ce qui correspond aux lectures les plus précises.

D. *Rapport* C/N

Le taux d'N est déterminé selon Kjeldahl.

III. RÉSULTATS EXPÉRIMENTAUX

Nous avons réuni dans le Tableau 1 les valeurs de la densité optique exprimée en pourcentages et nous avons groupé dans le Tableau 2 la densité optique à 490 mμ, les taux de coagulation à 30 et 40 me de Ca/l (à 20 me/l, seuls les acides des sols sont floculés; à 80 me/l tous les produits sauf n° 20 le sont au moins à 60–65%) et le rapport C/N.

Une analyse aussi sommaire d'un nombre restreint d'échantillons ne donne qu'un bien vague aperçu des produits très divers formés au

TABLEAU 1

Densités optiques des acides humiques										
Mode d'extraction	NaOH 0,1 *N*					NaF 1%				
Longueur d'onde, mμ	420	490	550	620	665	420	490	550	620	665
A(Podzol)	150	80	44	22,5	13	150	80	42	21,5	12
S(Mull)	130	76	39,5	22	12,5	150	88	44	25	13
R(Rendzine)	110	62	33	17,7	10,5	150	130	77	43	26
Ac. fulvique (Podzol)	—	—	—	—	—	27	10,3	5,5	3	2,2
D8 (Nov. 58, Avr. 59)	118	74	40	20	10,5	—	—	—	—	—
C14–P	80	45	27	15	6,5	—	85	57	33	19
C11–Dɛ–N	81	51	30	17	9,5	—	76	47	30	14
C15–W	64	37	24	14	8	83	48	30,5	17,5	10
L	53	29	18	10	6,5	—	60	37,5	23	14
U	34	17	9,7	6	3,2	76	46	28	16,5	9,2
Q	23	11	6,5	4	2,5	—	21	12,8	7,5	4,5
N° 2 + 3 — T	19,3	7,5	4	3	1,5	38	20	12	7	4,2
N° 3	—	—	—	—	—	23,7	11	5		2 *
M	68	35	21	11	6,5	—	68	42	25	14
N° 2 — K	54	30	18,5	10	6	—	—	—	—	—
X	37	18	10,5	5,2	3,2	60	32,5	20,5	11	6,5
N° 5	105	61	42	25	14	—	—	—	—	—

*: valeur à 650 mμ

cours de la décomposition du bois. Cependant nous pouvons faire certaines remarques:

1°) L'extraction à la soude fournit des produits de densité optique faible et de C/N élevé, sans doute parce qu'ils sont souillés d'impuretés, peut-être de nature glucidique comme dans le cas des tourbes [5]. Leurs caractéristiques n'en sont pas moins intéressantes, elles permettent un classement des produits qui concorde bien avec les indications des extraits NaF et, enfin, ce mode d'extraction fournit des quantités beaucoup plus élevées de produit. La chose est avantageuse quand on dispose de poids réduits de sciure, par exemple lors de l'analyse d'une fiole de culture.

2°) L'étude de la densité optique fournit, comme dans les sols, des résultats satisfaisants et permet de réunir les produits dont l'histoire et le degré de décomposition sont semblables. Ses indications concordent généralement bien avec celles que fournissent les deux autres critères.

3°) Parmi les produits sodiques, seul D8 est assimilable aux acides humiques des sols. D'ailleurs l'échantillon dont il provient paraît

TABLEAU 2

Mode d'extraction	NaOH 0,1 *N*					NaF 1%			
Caracteristiques	pH	Densité optique (490 mμ)	Coagulation par $CaCl_2$ 30 me/l	Coagulation par $CaCl_2$ 40 me/l	C/N	Densité optique (490 mμ)	Coagulation par $CaCl_2$ 30 me/l	Coagulation par $CaCl_2$ 40 me/l	C/N
A(Podzol)	3,2	80	49	58	25,5	80	60	65	—
S(Mull)	5,5	76	53	71	—	88	—	—	—
R(Rendzine) . . .	7,4	62	33	52	18,9	130	88	89	—
N	4,1	51	20	58	14,5	76	—	50	8
L	5,0	29	—	27	18	60	—	28	—
O	3,7	11	—	39	40	21	—	22	25
T	4,1	7,5	—	louche	46	20	—	louche	17,5
P	6,3	45	—	22	7,7	85	24	71	18
M	7,2	35	—	louche	15,5	68	—	62	—
K	5,8	30	—	louche	38	—	—	—	—
X	6,2	18	—	louche	30	32,5	—	forte	—
W	5,5	37	16	37	19	48	—	66	11
U	4,0	17	—	louche	29	46	52	59	19
n° 20	—	14,5	—	2	—	—	—	—	—
n° 5	—	61	—	52	—	—	—	—	—

avoir terminé son évolution et ressemble plus à un mull qu'à un compost de sciure.

Parmi les extraits NaF, celui de P est proche de ceux des horizons organiques A et S dont il diffère par une sensibilité plus réduite aux faibles concentrations de Ca. La différence est beaucoup plus grande entre les extraits sodiques: la sciure P n'est que partiellement transformée et tout se passe comme si la soude solubilisait des "impuretés" qui ne se retrouvent plus dans les sols.

Les autres acides humiques sont des intermédiaires allant de produits pâles parfois plus pâles même que les acides fulviques jusqu'au spécimen précédent (P).

4°) Nous savons dejà que la sciure peut subir deux évolutions différentes: l'une en milieu acide est jalonnée par T, O, L, N et *in vitro* par n° 3 et n° 20. Elle se caractérise par la teinte pâle des produits jeunes (T, O) et la faible sensibilité au Ca des substances extraites par NaF. Au contraire, les acides extraits par la soude sont plus facilement coagulés, à l'exception des sciures attaquées par Trechispora (T, U).

Enfin, au fur et à mesure que le bois dégradé et colonisé par de nouvelles populations s'assombrit, les caractéristiques des acides humiques s'améliorent et l'extrait NaF de N n'est plus tellement différent de celui de P malgré une aptitude encore plus réduite à la coagulation.

L'évolution neutre est représentée par X, K et surtout M; *in vitro* par n° 5 et n° 2. A degré de transformation égal, la densité optique est supérieure à celle des produits précédents et le comportement vis-à-vis de $CaCl_2$ est l'inverse de celui que nous venons de rencontrer: alors que les extraits sodiques ne floculent pratiquement pas, ceux au NaF sont très sensibles.

Ces deux évolutions se rencontrent côte à côte si l'on examine les échantillons W et U. Le premier parait indemne de lignivore; son pH est celui du bois frais, sa teneur en lignine atteint 37%; appartenant au deuxième type évolutif, son extrait NaF présente une sensibilité forte aux électrolytes et ceci malgré une teinte relativement pâle. Mais les couches supérieures ont été envahies à partir de T par Trechispora et revêtent aujourd'hui l'aspect d'une pourriture blanche: c'est l'échantillon U. Or, à en juger par les extraits NaF, cette transformation n'a guère modifié la densité optique des acides humiques tandis qu'elle abaissait leur degré de condensation

(sensibilité au Ca) ainsi que leur teneur en N. En même temps se formaient ces gommes extractibles par NaOH, floculables par les ions H^+ et non Ca^{++} et que nous avons déjà rencontrées dans les cultures pures de Trechispora [2]: les extraits sodiques ne floculent pratiquement plus par $CaCl_2$ et leur densité optique a diminué de plus de moitié.

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